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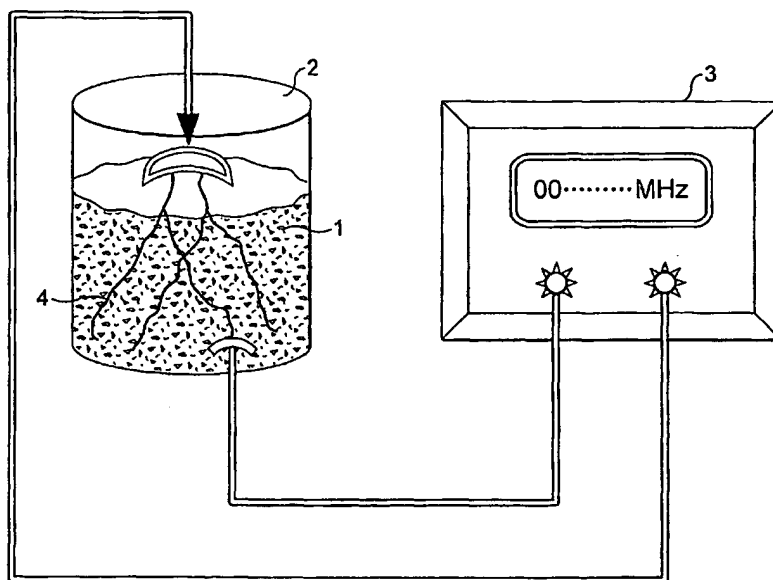
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[Continued on next page]

(54) Title: BIOLOGICAL COMPOSITIONS COMPRISING YEAST CELLS AND METHODS FOR TREATMENT OF CANCER



(57) Abstract: The present invention relates to biological compositions and dietary supplement comprising yeast cells that can produce a healthful benefit in a subject inflicted with cancer. The biological compositions can be used to retard the growth of cancer cells and/or prolonging the time of survival of the subject. The invention also relates to methods for manufacturing the biological compositions.

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BIOLOGICAL COMPOSITIONS COMPRISING YEAST CELLS AND METHODS FOR TREATMENT OF
CANCER**RELATED APPLICATIONS**

The present application claims priorities from U.S. patent applications
5 serial nos. 10/460,246, 10/460,247, 10/460,271, 10/460,323, 10/460,324, 10/460,325,
10/460,326, 10/460,327, 10/460,328, 10/460,336, 10/460,337, 10/460,338,
10/460,341, 10/460,437, 10/460,438, 10/460,530, 10/460,832, and 10/460,833, all
filed on June 11, 2003, the contents of which are herein incorporated by reference in
their entireties.

10 FIELD OF THE INVENTION

The invention relates to biological compositions comprising yeast cells
that can produce a healthful benefit in a subject inflicted with cancer. The invention
also relates to methods for manufacturing the biological compositions and methods of
use thereof.

15 BACKGROUND OF THE INVENTION**1. Cancer**

Cancer is one of the leading causes of death in animals and humans. It
is characterised primarily by an increase in the number of abnormal cells derived from
a given normal tissue. While surgery, chemotherapeutic agents and radiation are
20 useful in the treatment of cancer, there is a continued need to find better treatment
modalities and approaches to manage the disease that are more effective and less toxic
and non-invasive, especially when clinical oncologists are giving increased attention
to the quality of life of cancer patients. The present invention provides an alternative
approach to cancer therapy and management of the disease by using a biological
25 composition comprising yeasts.

2. Yeast-Based Compositions

Yeasts and components thereof have been developed to be used as
dietary supplement or pharmaceuticals. However, none of the prior methods uses
yeast cells which have been cultured in an electromagnetic field to produce a product

that has an anti-cancer effect. The following are some examples of prior uses of yeast cells and components thereof:

United States Patent No. 6,197,295 discloses a selenium-enriched dried yeast product which can be used as dietary supplement. The yeast strain
5 Saccharomyces boulardii sequela PY 31 (ATCC 74366) is cultured in the presence of selenium salts and contains 300 to about 6,000 ppm intracellular selenium. Methods for reducing tumor cell growth by administration of the selenium yeast product in combination with chemotherapeutic agents is also disclosed.

United States Patent No. 6,143,731 discloses a dietary additive
10 containing whole β -glucans derived from yeast, which when administered to animals and humans, provide a source of fiber in the diet, a fecal bulking agent, a source of short chain fatty acids, reduce cholesterol and LDL, and raises HDL levels.

United States Patent No. 5,504,079 discloses a method of stimulating an immune response in a subject utilizing modified yeast glucans which have
15 enhanced immunobiologic activity. The modified glucans are prepared from the cell wall of Saccharomyces yeasts, and can be administered in a variety of routes including, for example, the oral, intravenous, subcutaneous, topical, and intranasal route.

United States Patent No. 4,348,483 discloses a process for preparing a
20 chromium yeast product which has a high intracellular chromium content. The process comprises allowing the yeast cells to absorb chromium under a controlled acidic pH and, thereafter inducing the yeast cells to grow by adding nutrients. The yeast cells are dried and used as a dietary supplement.

Citation of documents herein is not intended as an admission that any
25 of the documents cited herein is pertinent prior art, or an admission that the cited documents are considered material to the patentability of the claims of the present application. All statements as to the date or representations as to the contents of these documents are based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of these
30 documents.

SUMMARY OF THE INVENTION

The present invention relates to biological compositions useful for treatment of subjects with cancer. In one embodiment, the present invention provides

biological compositions comprising live yeast cells which are capable of producing a healthful benefit in subjects with cancer. In other embodiments, the invention provides methods of making the biological compositions, and methods of using the biological compositions.

5 In particular, the methods of the invention comprise culturing yeast cells in the presence of a series of electromagnetic fields such that the yeast cells becomes metabolically active. The electromagnetic fields used are each defined by one of five frequency ranges and a broad range of field strength. The starting yeast cells are commercially available and/or accessible to the public, such as but not
10 limited to *Saccharomyces*. The methods for making the biological compositions of the invention may further comprise conditioning the activated yeast cells in plant extracts and the gastric juice of animals, while in the presence of another series of electromagnetic fields.

 The methods of manufacturing also comprise expanding the number of
15 activated or activated and conditioned yeast cells in large scale cultures in the presence of yet another series of electromagnetic fields, performing quality control measures, and packaging. Pharmaceutical compositions of the invention comprises activated and conditioned yeast cells and one or more pharmaceutically acceptable excipients or carriers. Additional ingredients, such as vitamins, herbs, and/or flavors
20 may be added to the biological compositions to form the oral compositions of the invention. Such additional carriers and ingredients can improve the healthful benefits, pharmacological properties, and organoleptic characteristics of the oral compositions. During the manufacturing process, the activated or activated and conditioned yeast cells may be dried and stored for a period of time.

25 The biological or oral compositions of the invention are ingested by the subject or used as an additive to be incorporated into food to be consumed by the subject. Dietary supplement and nutritional compositions comprising activated and conditioned yeast cells are encompassed by the invention. Preferably, the subject is a human being.

30 In various embodiments, the biological compositions of the invention are used to produce a healthful benefit in a subject with cancer or at high risk of developing cancer. In particular, the biological composition of the invention can retard the growth of cancer cells in an animal which received the composition orally.

The biological composition can also be used to prolong the time of survival of an animal with cancer.

BRIEF DESCRIPTION OF FIGURES

Fig. 1 Activation and conditioning of yeast cells. 1 yeast cell culture;
5 2 container; 3 electromagnetic field source; 4 electrode.

Fig. 2 Large scale propagation of yeast cells. 5 first container; 6 second container; 7 third container; 8 yeast cell cultures; 9 electromagnetic field source.

DETAILED DESCRIPTION OF THE INVENTION

10 1. General

The present invention relates to biological compositions that can produce a healthful benefit in a subject with cancer. The present invention provides methods for manufacturing the biological compositions as well as methods for using the biological compositions.

15 The term "cancer" as used herein refers to all types of cancers, or neoplasms or benign or malignant tumors, including but not limited to carcinoma, sarcoma, lymphoma, and leukemia. In particular, the term "cancer" as used herein includes but is not limited to lung cancer, nasopharyngeal cancer, esophageal cancer, stomach cancer, colorectal cancer, pancreatic cancer, liver cancer, testicular cancer,
20 prostate cancer, ovarian cancer, breast cancer, cervical cancer, uterine cancer, kidney cancer, bladder cancer, brain cancer, lymphoma, and leukemia.

In one embodiment, the invention provides biological compositions that comprise yeasts. Unlike the traditional use of yeasts in the making of food, the yeast cells of the invention are not used as a source of enzymes that acts on the food
25 ingredients. The yeasts are not a primary source of nutrients for the subject. Nor are yeast cells used as a carrier of an active ingredient, such as metal salts. The yeast cells of the invention are live when administered orally or ingested along with food by a subject. Without being bound by any theory or mechanism, the inventor believes that the culture conditions activate and/or amplified the expression of a gene or a set
30 of genes in the yeast cells such that the yeast cells become highly effective in stimulating the animal's immune system, including both specific and non-specific

immunological reactions, the results of which are manifested as the overall healthful benefits observed in the treated subject. The healthful benefits provided by using the biological compositions are demonstrated in animal models of human cancers, which show inhibition of tumor growth and prolonged survival time of animals with the
5 disease.

In another embodiment, the invention provides methods for making the yeast cells in the biological compositions. The starting materials are normal yeast cells which can be readily obtained commercially or from public microorganism deposits. The methods of the invention comprise a set of culture conditions that can
10 be applied reproducibly to activate the yeast cells. The key feature of the culture conditions used in the methods of the invention is a series of alternating electromagnetic fields of defined frequency ranges and field strengths which are applied to the growing yeast cell culture. The method further comprises the step of conditioning the activated live yeast cells to the acidic environment of the stomach of
15 the subject. The electromagnetic fields used in these methods can be created reproducibly at various scales, thus enabling even the large scale manufacturing of the biological compositions of the invention. By careful control of the culturing conditions, normal yeast cells can be activated routinely and reproducibly to become yeast cells of the invention.

In yet another embodiment, the invention provides methods for
20 manufacturing a biological composition comprising activated and conditioned yeasts of the invention, and additional ingredients, including but not limited to pharmaceutically acceptable carriers or excipients, vitamins, herbs (including traditional Chinese medicine products), herbal extracts, minerals, amino acids,
25 flavoring agents, coloring agents, and/or preservatives.

In yet another embodiment, the biological compositions can be added to food which will be consumed by the subject. As known to those skilled in the relevant art, many methods may be used to mix the biological or oral compositions of the invention with food while the yeast cells remain viable. In a particular
30 embodiment, the culture broth comprising live yeast cells of the present invention are added directly to food just prior to consumption. Dried powders of the yeasts can also be reconstituted and added directly to food just prior to consumption.

In various embodiments, the oral compositions of the invention can be consumed directly by a subject or be fed directly to a subject. For example, the

subject may drink the culture broth or a fraction thereof that comprises live activated and conditioned yeast cells. Oral compositions comprising dried yeast cells can also be given as a solid dosage form to the subject.

Although it is not necessary, the biological or oral compositions of the invention can be used in conjunction or in rotation with other types of treatment modalities such as but not limited to surgery, chemotherapeutic agents, and radiation. Since the biological compositions of the invention are administered orally, the assistance of health professionals in administration of the composition is generally not essential.

Described below are the yeast cells of the invention and methods of their preparation, followed by descriptions of using the biological compositions of the invention in a subject suffering from cancer. The examples of using the invention in treatment of eighteen (18) commonly occurring cancers are also provided to further demonstrate the therapeutic benefits of an oral composition of the invention. The activated and conditioned yeast cells in the oral composition are characterized by their ability to (i) suppress the growth of cancer cells in an animal model of human cancer or (ii) prolong the survival of animals with transplanted cancer cells in a model of human cancer as compared to yeast cells which have not been activated and conditioned.

2. Yeast Cells

The yeast cells of the biological composition are produced by culturing a plurality of yeast cells in an appropriate culture medium in the presence of an alternating electromagnetic field over a period of time. The method comprises a first step of activating the yeast cells and a second step of conditioning the activated yeast cells. The activation process comprises culturing yeast cells in the presence of at least two, three, four or five electromagnetic fields of specific frequencies and field strength. The conditioning process comprises further culturing of the activated yeast cells in a medium comprising plant extracts and extracts from the stomach of an animal, in the presence of at least one electromagnetic field. The activated and conditioned yeast cells can be stored as dried cells after drying the cells under appropriate conditions. The dried activated and conditioned yeast cells can be used later in large scale culturing processes for manufacturing the biological compositions

of the invention. The various culturing processes of the invention can be performed either as a batch process or a continuous process.

In various embodiments, yeasts of the genera of *Saccharomyces*, *Candida*, *Crebrothecium*, *Geotrichum*, *Hansenula*, *Kloeckera*, *Lipomyces*, *Pichia*,
 5 *Rhodospiridium*, *Rhodotorula*, *Torulopsis*, *Trichosporon*, and *Wickerhamia* can be used in the invention. Generally, fungi used for food manufacturing are preferred.

Non-limiting examples of yeast strains include *Saccharomyces sp.*, AS2.311; *Schizosaccharomyces pombe* Linder, AS2.214, AS2.248, AS2.249, AS2.255, AS2.257, AS2.259, AS2.260, AS2.274, AS2.994, AS2.1043, AS2.1149,
 10 AS2.1178, IFFI 1056; *Saccharomyces sake* Yabe, ACCC2045; *Saccharomyces uvarum* Beijer, IFFI 1023, IFFI 1032, IFFI 1036, IFFI 1044, IFFI 1072, IFFI 1205, IFFI 1207; *Saccharomyces rouxii* Boutroux, AS2.178, AS2.180, AS2.370, AS2.371; *Saccharomyces cerevisiae* Hansen Var. ellipsoideus, ACCC2043, AS2.2, AS2.3, AS2.8, AS2.53, AS2.163, AS2.168, AS2.483, AS2.541, AS2.559, AS2.606, AS2.607,
 15 AS2.611, AS2.612; *Saccharomyces carlsbergensis* Hansen, AS2.116, AS2.162, AS2.189, AS2.200, AS2.216, AS2.265, AS2.377, AS2.417, AS2.420, AS2.440, AS2.441, AS2.443, AS2.444, AS2.459, AS2.595, AS2.605, AS2.638, AS2.742, AS2.745, AS2.748, AS2.1042; *Rhodotorula aurantiaca* (Saito)Ladder; AS2.102, AS2.107, AS2.278, AS2.499, AS2.694, AS2.703, AS2.704 and AS2.1146;
 20 *Saccharomyces cerevisiae* Hansen, ACCC2034, ACCC2035, ACCC2036, ACCC2037, ACCC2038, ACCC2039, ACCC2040, ACCC2041, ACCC2042, AS2.1, AS2.4, AS2.11, AS2.14, AS2.16, AS2.56, AS2.69, AS2.70, AS2.93, AS2.98, AS2.101, AS2.109, AS2.110, AS2.112, AS2.139, AS2.173, AS2.182, AS2.196, AS2.242, AS2.336, AS2.346, AS2.369, AS2.374, AS2.375, AS2.379, AS2.380,
 25 AS2.382, AS2.393, AS2.395, AS2.396, AS2.397, AS2.398, AS2.399, AS2.400, AS2.406, AS2.408, AS2.409, AS2.413, AS2.414, AS2.415, AS2.416, AS2.422, AS2.423, AS2.430, AS2.431, AS2.432, AS2.451, AS2.452, AS2.453, AS2.458, AS2.460, AS2.463, AS2.467, AS2.486, AS2.501, AS2.502, AS2.503, AS2.504, AS2.516, AS2.535, AS2.536, AS2.558, AS2.560, AS2.561, AS2.562, AS2.576,
 30 AS2.593, AS2.594, AS2.614, AS2.620, AS2.628, AS2.631, AS2.666, AS2.982, AS2.1190, AS2.1364, AS2.1396, IFFI 1001, IFFI 1002, IFFI 1005, IFFI 1006, IFFI 1008, IFFI 1009, IFFI 1010, IFFI 1012, IFFI 1021, IFFI 1027, IFFI 1037, IFFI 1042, IFFI 1045, IFFI 1048, IFFI 1049, IFFI 1050, IFFI 1052, IFFI 1059, IFFI 1060, IFFI 1062, IFFI 1202, IFFI 1203, IFFI 1209, IFFI 1210, IFFI 1211, IFFI 1212, IFFI 1213,

IFFI 1215, IFFI 1221, IFFI 1224, IFFI 1247, IFFI 1248, IFFI 1251, IFFI 1270, IFFI 1277, IFFI 1289, IFFI 1290, IFFI 1291, IFFI 1292, IFFI 1293, IFFI 1297, IFFI 1300, IFFI 1301, IFFI 1302, IFFI 1307, IFFI 1308, IFFI 1309, IFFI 1310, IFFI 1311, IFFI 1331, IFFI 1335, IFFI 1336, IFFI 1337, IFFI 1338, IFFI 1339, IFFI 1340, IFFI 1345, IFFI 1348, IFFI 1396, IFFI 1397, IFFI 1399, IFFI 1441 and IFFI 1443. Preferred yeast strains include but are not limited to *S. cerevisiae* AS2.501, AS2.502, AS2.503, AS2.504, AS2.535, AS2.558, AS2.560, AS2.561 and AS2.562.

Generally, yeast strains useful for the invention can be obtained from private or public laboratory cultures, or publicly accessible culture deposits, such as the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 and the China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture Collection of Microorganisms, Institute of Microbiology, Chinese Academy of Sciences, Haidian, P.O. Box 2714, Beijing, 100080, China.

Non-limiting examples of using yeast cells of the invention are provided below. The yeast cells of the invention do not comprise an enhanced level of selenium or chromium relative to that found in naturally occurring yeast cells. In certain embodiments, the biological compositions do not comprise cells of *Saccharomyces boulardii* (for example, ATCC Accession No. 74366) or cells of a particular strain of *Saccharomyces cerevisiae* (strain Hansen CBS 5926) that is also commonly referred to as *Saccharomyces boulardii*.

Although it is preferred, the preparation of the yeast cells of the invention is not limited to starting with a pure strain of yeast. The yeast cells in the biological compositions may be produced by culturing a mixture of yeast cells of different species or strains. The constituents of a mixture of yeast cells can be determined by standard yeast identification techniques well known in the art.

In various embodiments of the invention, standard techniques for handling, transferring and storing yeasts are used. Although it is not necessary, sterile conditions or clean environments are highly desirable when carrying out the manufacturing processes of the invention, especially when the biological compositions are for human consumption. The manufacturing process can be adapted to meet regulatory guidelines on product safety and quality control by standard practice known in the art.

3. Electromagnetic Fields

As used herein, the terms "alternating electromagnetic field", "electromagnetic field" or "EM field" are synonymous. An electromagnetic field useful in the invention can be generated by various means well known in the art. A schematic illustration of exemplary setups are depicted respectively in Figure 1. An electromagnetic field of a desired frequency and a desired field strength is generated by an electromagnetic wave source (3) which comprises one or more signal generators that are capable of generating electromagnetic waves, preferably sinusoidal waves, and preferably in the frequency range of 1,500 to 15,000 MHz and most preferably 7,800 to 12,900 MHz. Such signal generators are well known in the art. Signal generators capable of generating signal with a narrower frequency range can also be used. If desirable, a signal amplifier can also be used to increase the output signal, and thus the strength of the EM field.

The electromagnetic field can be applied to the culture by a variety of means including placing the yeast cells in close proximity to a signal emitter connected to a source of electromagnetic waves. The signal generator is connected to the signal emitter by cables such as coaxial cables that can transmit signals up to greater than or equal to 30 GHz. Typically, the yeast cells are placed in a container which is made of material that is not an electric conductor, such as but not limited to plastic, resin, glass, and ceramic.

In one embodiment, the electromagnetic field is applied by signal emitters in the form of electrodes (4) that are submerged in a culture of yeast cells (1). In a preferred embodiment, one of the electrodes is a metal plate which is placed on the bottom of a non-conducting container (2), and the other electrode comprises a plurality of wires or tubes so configured inside the container such that the energy of the electromagnetic field can be evenly distributed in the culture. The electrodes are preferably made of copper. For an upright culture vessel, the tips of the wires or tubes are placed within 3 to 30 cm from the bottom of the vessel (i.e., approximately 2% to 10% of the height of the vessel from the bottom). Table 1 provides exemplary set up for culturing the yeast cells of the invention.

Table 1

Height of culture medium in the non-conducting	Distance electrodes are placed from the bottom	Range for distance of the electrodes from the
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container (cm)	of the container (cm)	bottom (cm)
15 to 20	3	3 to 5
20 to 30	5	5 to 7
30 to 50	7	7 to 10
50 to 70	10	10 to 15
70 to 100	15	15 to 20
100 to 150	20	20 to 30
150 to 200	30	25 to 30

The number of electrodes used depends on both the volume of the culture and the diameter of the electrode. For example, for a culture having a volume of 10 liter or less, two or three electrodes having a diameter of between 0.5 to 2.0 mm can be used. For a culture volume of 10 to 100 liter of culture, the electrodes can have a diameter of 3.0 to 5.0 mm. For a culture volume of 100 to 1,000 liter, the electrodes can have a diameter of 6.0 to 15.0 mm. For a culture having a volume greater than 1,000 liter, the electrodes can have a diameter of between 20.0 to 25.0 mm.

4. Activation of Yeast Cells

According to the invention, the method for producing activated yeast cells of the invention comprises culturing yeast cells in the presence of at least two, three, four or five alternating electromagnetic (EM) fields.

The culture process can be initiated by inoculating 1,000 ml of medium with an inoculum of a selected yeast strain (such as one of those described above) such that the starting cell density of the culture is greater than about 10^5 cells per ml. The starting culture can be used to seed larger scale culture. The culture is maintained initially at 28°C to 32°C for 22 to 30 hours prior to exposure to the EM field(s), typically at 30°C for 28 hours.

The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.08 mol/m³, preferably 0.04 mol/m³. The oxygen level can be controlled by any conventional means known in the art, including but not limited to stirring and/or bubbling.

The culture is most preferably carried out in a liquid medium which contains sources of nutrients assimilable by the yeast cells, such as sucrose or glucose, vitamin H, vitamin B₆, vitamin B₁₂, fetal calf serum, peptone, and inorganic ions such as K⁺, Na⁺, Mg²⁺, Ca²⁺, Cl⁻, CO₃²⁻, PO₄³⁻, and SO₄²⁻. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and is preferably between about 0.2% and 2%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium. Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are KH₂PO₄, (NH₄)₂HPO₄, CaCO₃, MgSO₄, NaCl, and CaSO₄.

In one embodiment, a medium containing one or more of sucrose or glucose (10-30 g), soluble starch (10 g), mannitol (10 g), vitamin A (40-60 µg), vitamin B₂ (40-50 µg), vitamin B₃ (40-60 µg), vitamin B₆ (30-80 µg), vitamin B₁₂ (30-60 µg), vitamin C (40-50 µg), vitamin D (20 µg), vitamin H (20-70 µg), bovine calf serum (30-35 ml), fetal calf serum (25-45 ml), KH₂PO₄ (0.20 g), MgSO₄·7H₂O (0.20-0.25 g), NaCl (0.20-0.30 g), CaSO₄·2H₂O (0.20-0.30 g), CaCO₃·5H₂O (3.0-4.0 g), peptone (2.5 g), and autoclaved water (up to 1,000 ml) can be used to culture the yeast cells of the invention. However, media containing ingredients other than the ones listed above can also be used in culturing the same or different strains of yeast cells. Preferably, the culturing medium is heated to 45°C and cooled before adding nutrients such as vitamin A, vitamin B₂, vitamin B₃, vitamin B₆, vitamin B₁₂, vitamin C, vitamin D, vitamin H, bovine calf serum and/or fetal calf serum.

It should be noted that the composition of the media provided herein is not intended to be limiting. The process can be scaled up or down according to needs. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

In certain embodiments, a series of at least two, three, four or five EM fields are applied to the culture of yeast cells, each having a different frequency

within a stated range, and a different field strength within a stated range. The EM fields can be applied in any order and by any means known in the art, such as the apparatus described above. Although any of the following two, three or four EM fields can be applied, preferably, all five EM fields are applied.

5 For the first EM field, the frequency is in the range of 7,821 to 10,170 MHz and the field strength is in the range of 200 to 350 mV/cm. The yeast culture is exposed to this first EM field at $30 \pm 2^\circ\text{C}$ for about 5 to 36 hours.

 For the second EM field, the frequency is in the range of 7,993 to 11,530 MHz and the field strength is in the range of 190 to 330 mV/cm. The yeast
10 culture is exposed to this second EM field at $30 \pm 2^\circ\text{C}$ for about 4 to 36 hours.

 For the third EM field, the frequency is in the range of 9,907 to 12,285 MHz and the field strength is in the range of 230 to 430 mV/cm. The yeast culture is exposed to this third EM field at $30 \pm 2^\circ\text{C}$ for about 10 to 34 hours.

 For the fourth EM field, the frequency is in the range of 11,141 to
15 12,842 MHz and the field strength is in the range of 220 to 450 mV/cm. The yeast culture is exposed to this fourth EM field at $30 \pm 2^\circ\text{C}$ for about 4 to 34 hours.

 For the fifth EM field, the frequency is in the range of 12,031 to 12,900 MHz and the field strength is in the range of 260 to 450 mV/cm. The yeast culture is exposed to this fifth EM field at $30 \pm 2^\circ\text{C}$ for about 5 to 34 hours.

20 In less preferred embodiments, the yeast cells can be cultured by exposure to two, three or four of the above-mentioned EM fields in a different order. The yeast cells can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

25 The cell density of the culture at the end of the activation process is typically greater than about 10^6 to 10^9 cells per ml (estimated by hemacytometer). The activated yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C to 4°C . The activated yeast cells recovered from the liquid culture may be dried and stored in powder form.
30 Preferably, the powder form of the yeast cells comprises greater than about 10^7 to 10^{10} yeast cells per gram.

5. Conditioning of Yeast Cells

According to the invention, performance of the activated yeast cells can be optimized by culturing the activated yeast cells in the presence of an extract from the stomach (e.g., the gastric juice) of an animal with physiology similar to the subject to which the biological composition will be administered. The inclusion of
5 this additional conditioning process allows the activated yeast cells to adapt to and endure the acidic environment of the subject's stomach. The method for conditioning activated yeast cells of the invention comprises culturing yeast cells in such materials in the presence of at least one EM field.

The culture process can be initiated by inoculating 1,000 ml of a
10 conditioning medium with about 10 gram of dried activated yeasts containing about 10^{10} cells per gram (as prepared by the methods described above). An equivalent number of yeast cells in culture, preferably greater than 10^6 to 10^9 cells per ml, more preferably at 10^8 cells per ml, can also be used as an inoculum. The conditioning medium comprises per 1,000 ml about 700 ml of gastric juice of an animal and about
15 300 ml of wild hawthorn juice. The process can be scaled up or down according to needs.

The gastric juice of an animal can be obtained from the stomach content of a freshly slaughtered animal. Although not essential, the animal is preferably kept under a clean environment, and fed a standard diet, preferably germ-free. For example, the content of the stomach of a 120-day old pig is mixed with
20 2,000 ml of distilled water, and allowed to settle without stirring for 6 hours. The clear liquid above is collected for use as the gastric juice used in the conditioning process. The gastric juice of a pig can be used to condition yeast cells for use in a variety of mammals, including humans. Other methods that can be used to collect the
25 gastric juice include centrifugation or filtration of the mixture to remove debris and/or microorganisms. The gastric juice so obtained can be stored at 4°C. Preferably, the collection procedures and storage are carried out under sterile conditions.

The wild hawthorn juice is an extract of wild hawthorn fruits prepared by slicing the fruits and drying the slices in air, preferably to less than 8% moisture
30 (commercial dryer can be used if necessary), crushing the dried fruits to less than 20 mesh, and mixing 1,500 ml of water per 500 gram of the crushed wild hawthorn. The mixture is then allowed to settle without stirring for 6 hours, and the clear liquid above is collected for use as the wild hawthorn juice used in the conditioning process. Other methods that can be used to collect the hawthorn juice include centrifugation or

filtration of the mixture. Preferably, the collection procedures and storage are carried out under sterile conditions.

The activated yeast cells are conditioned by culturing in at least one of the following two EM fields which can be applied by the apparatus described above
5 or any means known in the art:

The first EM field has a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 230 to 440 mV/cm. The temperature is maintained at 28°C to 32°C, and typically at 30°C. The yeast culture is exposed to this first EM field for about 4 to 50 hours.

10 The second EM field has a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm. The temperature is maintained at 28°C to 32°C, and typically at 30°C. The yeast culture is exposed to this second EM field for 10 to 52 hours.

In a preferred embodiment, the activated yeast cells are conditioned by
15 culturing in both of the above-mentioned EM fields. In less preferred embodiments, the yeast cells are conditioned in the two different EM fields in a different order. In other embodiments, a series of EM fields having field characteristics within the ranges stated above can be applied to condition the yeast cells. The yeast cells can remain in the same container and use the same set of electromagnetic wave generator
20 and emitters when switching from one EM field to another EM field.

The cell density of the culture at the end of the activation and conditioning process is typically greater than about 10^7 to 10^{10} cells per ml (estimated by hemacytometer). The activated and conditioned yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature
25 below about 0°C to 4°C.

The activated and conditioned yeast cells can be used directly in a biological composition or used as a starter culture for large scale manufacturing. The activated and conditioned yeast cells recovered from the liquid culture may be dried and stored in powder form. Preferably, the powder form of the activated and
30 conditioned yeast cells comprises greater than about 10^8 to 10^{11} yeast cells per gram.

6. Large Scale Manufacturing

The present invention also encompasses methods of manufacturing of the biological compositions of the invention at a large scale. The activated and

conditioned yeast cells as prepared above are propagated on a large scale to make the biological compositions of the invention. The method comprises culturing the yeast cells in the presence of one or more EM fields for a period of time, diluting the growing yeast cells with fresh medium, and repeating the process. The method can be
5 carried out as a batch process or a continuous process.

In one preferred embodiment, a set of three containers (5, 6, 7) each comprising a set of electrodes for generating an electromagnetic field as described above are set up each with 1,000 liters of a culture medium. See Figure 2. The culture medium comprises nutrients assimilable by the yeast cells as shown in Table
10 2.

Table 2

Material	Quantity
Wild hawthorn juice	300 liters
Jujube juice	300 liters
Wu wei zi juice	300 liters
Soybean juice	100 liters

The wild hawthorn juice is an extract of fresh wild hawthorn fruits prepared by washing the fruits clean, drying the fruits in air or using a commercial
15 dryer to less than 8% moisture, crushing the dried fruits to less than 20 mesh, and mixing the crushed wild hawthorn with water at a ratio of 400 liters of water per 100 kg of crushed fruits. The mixture is then stirred continuously for 12 hours while the temperature is maintained at 28°C to 30°C. The mixture is then centrifuged at 1,000 rpm to collect the supernatant which is used as described above. Preferably, the
20 procedures are carried out under sterile conditions.

The jujube juice is an extract of fresh jujube fruits prepared by washing the fruits clean, drying the fruits to less than 8% moisture, crushing the dried fruits to less than 20 mesh, and mixing the crushed jujube with water at a ratio of 400 liters of water per 100 kg of crushed fruits. The mixture is then stirred continuously for 12
25 hours while the temperature is maintained at 28°C to 30°C. The mixture is then

centrifuged at 1,000 rpm to collect the supernatant which is used as described above. Preferably, the procedures are carried out under sterile conditions.

The wu wei zi juice is an extract of fresh berries of *Schisandra chinensis* plant prepared by washing the berries, drying the fruits to less than 8% moisture, crushing the dried berries to less than 20 mesh, and mixing the crushed berries with water at a ratio of 400 liters of water per 100 kg of crushed berries. The mixture is then stirred continuously for 12 hours while the temperature is maintained at 28°C to 30°C. The mixture is then centrifuged at 1,000 rpm to collect the supernatant which is used as described above. Preferably, the procedures are carried out under sterile conditions.

The soybean juice is prepared by washing the soybeans, drying the soybeans to less than 8% moisture, crushing the soybeans to less than 20 mesh, and mixing the crushed soybeans with water. For 30 kg of soybeans, 130 liters of water is used. The mixture is then stirred continuously for 12 hours while the temperature is maintained at 28°C to 30°C. The mixture is then centrifuged at 1,000 rpm to collect the supernatant which is used as described above. Preferably, the procedures are carried out under sterile conditions.

The first container is inoculated with activated or activated and conditioned yeast cells as prepared by the methods as set forth above. About 1,000 gram of dried yeast powder are added to 1,000 liter of culture medium. Each gram of the dried yeast powder comprises about 10^{10} yeast cells. Instead of dried yeast cells, an equivalent number of yeast cells in a liquid medium can also be used, preferably greater than about 10^6 to 10^9 cells per ml, more preferably about 10^7 cells per ml.

The yeast cells in the first container (5) are then subjected to a series of two EM fields. For the first EM field, which can be applied by the apparatus described above, the frequency is in the range of 11,141 to 12,842 MHz and the field strength is in the range of 170 to 450 mV/cm. The yeast culture is exposed to this first EM field for about 4 to 25 hours. The yeast cells are then subjected to a second EM field having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 120 to 450 mV/cm. The yeast culture is exposed to this second EM field for about 4 to 24 hours. The yeast cells from the first container are then transferred to the second container which contains about 1,000 liter of the culture medium. In effect, the first yeast culture is diluted by about 50% with fresh culture medium.

In the second container (6), the yeast cells are again subjected to a series of two EM fields. The frequencies used in the second container are similar to those used in the first container. The yeast cells from the second container are then transferred to the third container which contains yet another 1,000 liter of the culture medium. Again, the second yeast culture is diluted by about 50% with fresh culture medium.

In the third container (7), the yeast cells are again subjected to a series of two EM fields. The frequencies used in the third container are similar to those used in the first and second container.

The yeast cell culture resulting from the end of this stage can be used directly as an oral composition of the invention, or used to form other compositions encompassed by the invention.

The cell density of the culture at the end of the large scale manufacturing process is typically greater than about 10^8 to 10^{10} cells per ml (estimated by hemacytometer). The concentration of yeast cells in the medium can be concentrated or diluted accordingly. In certain embodiments, the concentration of yeast cells in the medium is in the range of 10^3 to 10^{10} cells per ml. In less preferred embodiments, the concentration of yeast cells in the medium is in the range of 10^3 to 10^6 cells per ml. In more preferred embodiments, the concentration of yeast cells in the medium is greater than 10^6 to 10^{10} cells per ml. In most preferred embodiments, the concentration of yeast cells in the medium is in the range of 10^6 to 5×10^8 cells per ml.

Other ingredients that enhance the healthful benefits, pharmacological properties and/or organoleptic characteristics of the composition can be added to the yeast cell culture. To maintain viability and freshness of the composition, it is preferred that the various downstream and packaging process be carried out below room temperature, and preferably at 0°C to 4°C . In one embodiment, the yeast cell culture can be packaged in liquid containers.

In another embodiment, the activated and conditioned yeast cells can be dried as follows. The yeast cell culture is first centrifuged under 75 to 100 g for 10 to 20 minutes to remove the supernatant. The residue which may contain up to 85% moisture is dried in a first dryer at a temperature not exceeding $60 \pm 2^\circ\text{C}$ for a period of 5 minutes so that yeast cells quickly became dormant. The yeast cells were then sent to a second dryer and dried at a temperature not exceeding $65 \pm 2^\circ\text{C}$ for a period

of about 8 minutes to further remove at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% of water. For example, the yeast cells may be dried to remove at least 88% of water so the dried yeast cells may contain up to 12% moisture.

5 After cooling to room temperature, the dried yeast cells can be packaged by standard pharmaceutical methods in various solid dosage form, each containing a predetermined amount of the dried material. In a preferred embodiment, the dried material comprises about 10^5 to 10^{11} cells per gram. In a more preferred embodiment, the dried material comprises about 10^8 to 5×10^{10} cells per gram. In a
10 most preferred embodiment, the dried material comprises about 5×10^8 cells per gram.

In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers.

7. Methods of Use

15 The present invention further provides methods of use of the biological compositions of the invention. In one embodiment, the biological composition is used as a medicament for treatment of cancer. In another embodiment, the biological composition is used as a dietary supplement, health food, or health drink. The methods comprise administering an effective amount of the biological composition to
20 a subject in need. The biological composition may be administered orally, in liquid or solid form, or enterally through a feeding tube. As used herein, the term "an effective amount" means an amount sufficient to provide a therapeutic or healthful benefit in the context of cancer.

 According to the invention, the biological composition can produce a
25 healthful benefit in a subject suffering from cancer. The subject is preferably a mammal such as a non-primate (*e.g.*, a cow, pig, horse, cat, dog, rat, mouse, rabbit, etc.) or a primate (*e.g.*, a monkey, chimpanzee, human, etc.). In one embodiment, the subject is non-human. Most preferably, the subject is a human being.

 In one embodiment, the subject in need is one who is diagnosed with
30 cancer with or without metastasis, at any stage of the disease (*e.g.*, TNM staging by the American Joint Committee for Cancer (AJCC) published in 1988, or other staging system acceptable in the art). The subject may be a cancer patient who is receiving concurrently other treatment modalities against the cancer. The subject can be a

cancer patient who had undergone a regimen of treatment (e.g., chemotherapy and/or radiation) and whose cancer is regressing. The subject may be a cancer patient who had undergone a regimen of treatment (e.g., surgery) and who appears to be clinically free of the cancer. The biological composition of the invention can be administered

5 adjunctively with any of the treatment modalities, such as but not limited to chemotherapy, radiation, and/or surgery. For example, the biological composition can be used in combination with one or more chemotherapeutic or immunotherapeutic agents, such as anastrozole (Arimidex®), amsacrine (AMSA), L-asparaginase (Elspar®), bleomycin, bleomycin sulfate (Blenoxane®), busulfan (Myleran®),

10 carboplatin (Paraplatin®), carmustine (BCNU®, Gliadel®, BiCNU®), celecoxib (Celebrex®), cetuximab (IMC-C225 or Erbitux™), cisplatin (platinum analogs, Platinol®), chlorambucil (Leukeran®), cladribine (2-chlorodeoxyadenosine; “2-CDA”; Leustatin®), cyclophosphamide (Cytoxan®), cyclosporin A, cytarabine (ara-C; Cytosar-U®), daunorubicin (Cerubidine®), dexamethasone, docetaxel, doxorubicin

15 (Doxil®, Rubex® or Adriamycin®), epirubicin (Pharmorubicin®), estramustine, estramustine phosphate (Emcyt®), etoposide (VePesid® or VP-16®), exemestane (Aromasin®), floxuridine (FUDR®), 5-fluorouracil, fludarabine phosphate (Fludara®), gemcitabine HCL (Gemzar®), hydroxyurea (Hydrea®), hexamethylmelamine, idarubicin (Idamycin®), ifosamide (Ifex®), alfa-2a interferon,

20 irinotecan (CPT-11, or Camptosar®), letrozole (Femara®), leucovorin, megestrol (Megace®), methotrexate, methotrexate sodium plus 6-mercaptopurine (6-MP; Purinethol®), mitomycin C, mitoxantrone (Novantrone®), nitrosureas, oxaliplatin, oxorubicin, paclitaxel (Taxol®, Taxotere®, Tamoxifen®, Nolvadex®), pentostatin (2-deoxycoformycin; “DCF”; Nipent®), prednisone, procarbazine, raloxifene

25 (Evista®), raltitrexed (Tomudex®), retinoic acid (ATRA), temozolomide (Temodar®), 6-thioguanine (Tabloid®), thiotepa (Thioplex®), topotecan (Hycamtin®), toremifene (Fareston®), trastuzumab (Herceptin®), valrubicin (Valstar™), vinblastine, vincristine, vincristine sulfate (Oncovin®), and/or vinblastine sulfate (Velban®). The biological composition can also be used after other

30 regimen(s) of treatment is concluded.

The subject may be one who has not yet been diagnosed with cancer but are predisposed to or at high risk of developing as a result of genetic factors and/or environmental factors. The subject may also be one who displays

characteristics that are associated with a high risk of cancer such as nodules detected by computer tomographic scanning or suspect cells in biopsy and/or body fluids.

Depending on the subject, the therapeutic and healthful benefits range from inhibiting or retarding the growth of the cancer and/or the spread of the cancer to
5 other parts of the body (i.e., metastasis), palliating the symptoms of the cancer, improving the probability of survival of the subject with the cancer, prolonging the life expectancy of the subject, improving the quality of life of the subject, and/or reducing the probability of relapse after a successful course of treatment (e.g., surgery, chemotherapy or radiation).

10 In particular, the invention provides a method for retarding the growth of cancer cells in a subject, such as a human, comprising administering orally to the subject a biological composition of the invention. The invention also provide a method for prolonging the time of survival of a subject inflicted with cancer preferably a human patient, comprising administering orally to the subject a biological
15 composition of the invention.

The effective dose will vary with the subject treated. The effective dose for the subject will also vary with the condition to be treated and the severity of the condition to be treated. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual subject. In general,
20 the total daily dose range of activated and conditioned yeast cells for a subject inflicted with cancer is from about 10^5 to 10^{11} cells per day; preferably, about 10^8 to 5×10^{10} cells per day; more preferably, about 2×10^9 cells per day in powder form or 9×10^8 to 1×10^{10} cells per day in liquid preparations, administered in single or divided doses orally. The length of time for a course of treatment should be at least 1 week, at
25 least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 7 weeks, at least 10 weeks, at least 13 weeks, at least 15 weeks, at least 20 weeks, at least 6 months, or at least 1 year. It may be necessary to use dosages outside these ranges in some cases as will be apparent to those skilled in the art. In certain embodiments, the oral compositions can be administered for a period of time until the symptoms and/or
30 infection of the patients by the bacteria and viruses are under control, or when the disease has regressed partially or completely. For use as a dietary supplement, the total daily dose range should be from about 10^5 to 10^{11} cells per day; preferably, about 5×10^7 to 5×10^9 cells per day. The oral compositions can be administered as a dietary supplement for as long as 6 months, or in accordance with recommended

length of use under the Dietary Supplement Health and Education Act (DSHEA) or other government or industry guidelines. Further, it is noted that the nutritionist, dietician, clinician or treating physician will know how and when to interrupt, adjust, or terminate use of the biological composition as a medicament or dietary supplement
5 in conjunction with individual patient response.

The effect of the biological compositions of the invention on development and progression of cancer can be monitored by any methods known to one skilled in the art, including but not limited to measuring: a) changes in the size and morphology of the tumor using imaging techniques such as a computed
10 tomographic (CT) scan or a sonogram; and b) changes in levels of biological markers of risk for cancer.

8. Formulations

The biological compositions of the present invention comprise activated and conditioned live yeast cells prepared as described above, as active
15 ingredient, and can optionally contain a pharmaceutically acceptable carrier or excipient, and/or other ingredients provided that these ingredients do not kill or inhibit the yeast cells. Other ingredients that can be incorporated into the biological compositions of the present invention, may include, but are not limited to, herbs (including traditional Chinese medicine products), herbal extracts, vitamins, amino
20 acids, metal salts, metal chelates, coloring agents, flavor enhancers, preservatives, and the like.

Any dosage form may be employed for providing the subject with an effective dosage of the oral composition. Dosage forms include tablets, capsules, dispersions, suspensions, solutions, and the like. In one embodiment, compositions of
25 the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, or tablets, each containing a predetermined amount of activated and conditioned yeast cells, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion. In general, the compositions are prepared by uniformly
30 and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation. Such products can be used as pharmaceuticals or dietary supplements, depending on the dosage and circumstances of its use.

The oral compositions of the present invention may additionally include binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); binders or fillers (e.g., lactose, pentosan, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g.,
5 magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets or capsules can be coated by methods well known in the art.

Liquid preparations for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product
10 for constitution with water or other suitable vehicle before use. The temperature of the liquid used to reconstitute the dried product should be less than 65°C. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia);
15 non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). As described below, the preparations can also be made to resemble foods or beverages, containing buffer salts, flavoring, coloring and sweetening agents as appropriate. In certain embodiments, the oral composition is a cell suspension
20 comprising about 10^3 to 10^{10} cells per ml. The oral composition can be produced by diluting or concentrating the yeast culture medium produced by the method set forth herein. In less preferred embodiments, the oral composition is a cell suspension containing about 10^3 to 10^6 cells per ml. In more preferred embodiments, the oral composition is a cell suspension containing greater than about 10^6 to 10^{10} cells per ml.
25 In most preferred embodiments, the oral composition is a cell suspension containing about 10^6 to 5×10^8 cells per ml. The oral composition can be formulated as a health drink and packaged in liquid containers, each containing a predetermined amount of the liquid yeast culture. Standard methods of quality control and packaging are applied to produce in one embodiment of the invention, oral compositions packaged
30 in liquid containers each comprising about 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 10 ml, 15 ml, 20 ml, 30 ml, 40 ml, 50 ml, 75 ml, 100 ml, 150 ml, 200 ml, 250 ml, 500 ml, 750 ml, or 1,000 ml of the live yeast cells. The number of container to be taken each day to obtain the total daily dose in a subject depends on the number of activated and conditioned yeast cells contained within each container. For example, a container

may comprise 50 ml of liquid with 10^7 cells per ml and when a total daily dose of about 2×10^9 cells per day is desired, a subject can drink 4 containers per day to obtain the desired total daily dose.

Generally, because of their ease of administration, tablets and capsules
5 represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers as described above are employed. In a preferred embodiment, the composition is a capsule. The capsules can be formulated by any commercially available methods. In certain embodiments, the composition is a capsule containing 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 40 mg, 50 mg, 75 mg, 100 mg, 150 mg, 200
10 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1.0 gram, 1.25 gram, 1.5 gram, or 2.0 gram of live yeast cells in powder form. The powder in the capsule comprises about 10^5 to about 10^{11} cells per gram; more preferably, about 10^8 to 5×10^{10} cells per gram; and most preferably, about 5×10^8 cells per gram. The number of capsule to be taken each day to obtain the total daily dose in a subject
15 depends on the number of activated and conditioned yeast cells contained within each capsule. For example, a capsule may comprise about 500 mg of powder with 5×10^8 cells per gram. To achieve a total daily dose of about 2×10^9 cells per day, a subject can take two capsules at a time for four times per day.

In another embodiment, the biological compositions comprising
20 activated and conditioned yeast cells can be added directly to foods so that an effective amount of yeast cells is ingested during normal meals. Any methods known to those skilled in the art may be used to add to or incorporate the biological compositions into natural or processed foods, provided that the activated and conditioned yeast cells remain viable. Preferably, the nutritional compositions of the
25 invention are made and stored under conditions, such as temperature, from about 0°C to 4°C . As used herein, the term "food" broadly refers to any kind of material, liquid or solid, that is used for nourishing an animal, and for sustaining normal or accelerated growth of an animal including humans. Many types of food products or beverages, such as but not limited to, fruit juice, herbal extracts, tea-based beverages,
30 dairy products, soybean product (e.g., tofu), and rice products, can be used to form nutritional compositions comprising the activated and conditioned yeast cells of the invention.

9. Preferred Embodiments

The invention can be further defined by reference to the following preferred embodiments. The use of the current invention for treatment of eighteen (18) commonly occurring cancers is set forth in detail below. However, it is to be understood that these embodiments are provided for illustrative purpose only and thus should not be interpreted to limit the scope of the current invention.

Strains of Yeast Cells

The following yeast cell strains were used to prepare the biological compositions that were administered to the animals used in the respective human cancer type models.

Table 3 Exemplary Strains of Yeast Cells for Treatment of Cancers

Cancer Type Model	Yeast Cell Strain
Lung Cancer	<i>Saccharomyces cerevisiae</i> Hansen strain IFFI1345
Nasopharyngeal Cancer	<i>Saccharomyces carlsbergensis</i> Hansen strain AS2.116
Esophageal Cancer	<i>Saccharomyces cerevisiae</i> Hansen strain AS2.375
Stomach Cancer	<i>Saccharomyces cerevisiae</i> Hansen strain AS2.14
Colorectal Cancer	<i>Saccharomyces cerevisiae</i> Hansen strain AS2.1396
Pancreatic Cancer	<i>Saccharomyces cerevisiae</i> Hansen strain IFFI 1413
Liver Cancer	<i>Saccharomyces cerevisiae</i> Hansen strain AS2.503
Testicular Cancer	<i>Saccharomyces cerevisiae</i> Hansen strain AS2.182
Prostate Cancer	<i>Saccharomyces carlsbergensis</i> Hansen strain AS2.440
Ovarian Cancer	<i>Saccharomyces cerevisiae</i> Hansen strain AS2.502
Breast Cancer	<i>Saccharomyces carlsbergensis</i> Hansen strain AS2.441
Cervical Cancer	<i>Saccharomyces carlsbergensis</i> Hansen strain AS2.444
Uterine Cancer	<i>Saccharomyces carlsbergensis</i> Hansen strain AS2.605
Kidney Cancer	<i>Saccharomyces carlsbergensis</i> Hansen strain AS2.189
Bladder Cancer	<i>Saccharomyces cerevisiae</i> Hansen strain AS2.4
Brain Cancer	<i>Saccharomyces cerevisiae</i> Hansen strain AS2.501
Lymphoma	<i>Saccharomyces cerevisiae</i> Hansen strain AS2.562
Leukemia	<i>Saccharomyces cerevisiae</i> Hansen strain AS2.11

Activation of Yeast Cells

The following electromagnetic (EM) fields can be used to activate the yeast cells to prepare the biological compositions of the invention. The yeast cells as set forth above in Table 3 were cultured in a liquid medium comprising one or more of sucrose or glucose (10-30 g), soluble starch (10 g), mannitol (10 g), vitamin A (40-60 µg), vitamin B₂ (40-50 µg), vitamin B₃ (40-60 µg), vitamin B₆ (30-80 µg), vitamin B₁₂ (30-60 µg), vitamin C (40-50 µg), vitamin D (20 µg), vitamin H (20-70 µg),

- bovine calf serum (30-35 ml), fetal calf serum (25-45 ml), KH_2PO_4 (0.20 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.20-0.25 g), NaCl (0.20-0.30 g), $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (0.20-0.30 g), $\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$ (3.0-4.0 g), peptone (2.5 g), and autoclaved water (up to 1,000 ml), and were subjected to a series of at least two, three, four, or five EM fields as set forth
- 5 below in Table 4 (numbers shown in parentheses are the preferred embodiments) in accordance with the general procedures set forth in the Detailed Description of the Invention.

Table 4

Cancer Type Model	1 st EM Field	2 nd EM Field	3 rd EM Field	4 th EM Field	5 th EM Field
Lung Cancer	8046-8056 (8051) MHz, 240-300 (293)mV/cm, about 5-25 (15) hrs	8077-8087 (8082) MHz, 226-285 (272)mV/cm, about 16-36 (26) hrs	9907-9917 (9912)MHz, 325-366 (354)mV/cm, about 13-33 (23) hrs	12716-12726 (12721)MHz, 386-412 (398)mV/cm, about 14-34 (24) hrs	12751-12760 (12755) MHz, 295-315 (302)mV/cm, about 5-25 (15) hrs
Nasopharyngeal Cancer	9961-9970 (9963) MHz, 250-270 (246)mV/cm, about 12-32 (22) hrs	10951-10960 (10956) MHz, 250-270 (250)mV/cm, about 5-15 (10) hrs	12091-12100 (12098)MHz, 250-270 (263)mV/cm, about 14-34 (24) hrs	12441-12450 (12446)MHz, 240-270 (244)mV/cm, about 5-15 (10) hrs	12750-12760 (12757) MHz, 270-290 (276)mV/cm, about 14-34 (24) hrs
Esophageal Cancer	7841-7850 (7846) MHz, 260-280 (272)mV/cm, about 10-30 (19.5) hrs	8921-8930 (8926) MHz, 250-270 (262)mV/cm, about 4-12 (8) hrs	10191-10200 (10192)MHz, 290-310 (293)mV/cm, about 14-34 (24) hrs	11241-11250 (11248)MHz, 270-290 (280)mV/cm, about 5-15 (10) hrs	12651-12660 (12652) MHz, 310-330 (317)mV/cm, about 14-34 (24) hrs
Stomach Cancer	7981-7990 (7987) MHz, 280-300 (286)mV/cm, about 11-31 (21) hrs	9131-9140 (9137) MHz, 280-300 (281)mV/cm, about 4-12 (8) hrs	10171-10180 (10178)MHz, 300-320 (312)mV/cm, about 12-32 (22) hrs	11221-11230 (11224)MHz, 300-320 (310)mV/cm, about 5-15 (10) hrs	12166-12175 (12171) MHz, 320-340 (332)mV/cm, about 10-30 (20) hrs
Colorectal Cancer	7941-7950 (7948) MHz, 230-250 (231)mV/cm, about 14-34 (24) hrs	9131-9140 (9135) MHz, 220-240 (221)mV/cm, about 5-15 (10) hrs	9991-10000 (9997)MHz, 250-270 (254)mV/cm, about 12-32 (22) hrs	11141-11150 (11148)MHz, 230-250 (233)mV/cm, about 5-15 (10) hrs	12771-12780 (12778) MHz, 260-280 (266)mV/cm, about 12-32 (22) hrs
Pancreatic Cancer	7961-7970 (7967) MHz, 250-270 (264)mV/cm,	10181-10190 (10188) MHz, 250-270 (266)mV/cm,	12276-12285 (12281)MHz, 270-290 (279)mV/cm,	12461-12470 (12466)MHz, 280-300 (286)mV/cm,	12761-12770 (12764) MHz, 300-320 (306)mV/cm,

	about 12-32 (22) hrs	about 5-15 (10) hrs	about 14-34 (24) hrs	about 5-15 (10) hrs	about 8-28 (18) hrs
Liver Cancer	7971-7980 (7976) MHz, 330-350 (335)mV/cm, about 10-30 (20) hrs	8841-8850 (8845) MHz, 300-320 (310)mV/cm, about 5-15 (10) hrs	10151-10160 (10152)MHz, 390-430 (422)mV/cm, about 14-34 (24) hrs	12121-12130 (12126)MHz, 400-420 (416)mV/cm, about 5-15 (10) hrs	12851-12860 (12854) MHz, 420-450 (445)mV/cm, about 14-34 (24) hrs
Testi- cular Cancer	8021-8030 (8028) MHz, 310-330 (327)mV/cm, about 14-34 (24) hrs	9011-9020 (9016) MHz, 310-330 (326)mV/cm, about 5-15 (10) hrs	9936-9945 (9941)MHz, 355-375 (365)mV/cm, about 14-34 (24) hrs	12061-12070 (12066)MHz, 355-375 (350)mV/cm, about 5-15 (10) hrs	12731-12740 (12737) MHz, 380-400 (387)mV/cm, about 14-34 (24) hrs
Prostate Cancer	10161-10170 (10164) MHz, 230- 250 (239)mV/cm, about 12-32 (22) hrs	11211-11220 (11218) MHz, 220-240 (232)mV/cm, about 5-15 (10) hrs	12176-12185 (12181)MHz, 250-270 (258)mV/cm, about 12-32 (22) hrs	12536-12545 (12541)MHz, 250-270 (251)mV/cm, about 5-15 (10) hrs	12656-12665 (12661) MHz, 280-300 (283)mV/cm, about 14-34 (24) hrs
Ovarian Cancer	10081-10090 (10088)MHz, 230-250 (243)mV/cm, about 10-30 (20) hrs	11210-11220 (11214) MHz, 210-230 (228)mV/cm, about 4-12 (8) hrs	12141-12150 (12142)MHz, 245-265 (257)mV/cm, about 12-32 (22) hrs	12341-12350 (12346)MHz, 220-240 (216)mV/cm, about 4-12 (8) hrs	12781-12790 (12786) MHz, 280-300 (283)mV/cm, about 14-34 (24) hrs
Breast Cancer	8061-8070 (8066) MHz, 220-240 (236)mV/cm, about 12-32 (22) hrs	10121-10130 (10124) MHz, 210-230 (226)mV/cm, about 4-12 (8) hrs	12241-12250 (12246)MHz, 255-275 (264)mV/cm, about 10-30 (20) hrs	12351-12360 (12355)MHz, 260-280 (275)mV/cm, about 4-12 (8) hrs	12791-12800 (12798) MHz, 270-290 (277)mV/cm, about 12-32 (22) hrs
Cervical Cancer	7821-7830 (7825) MHz, 230-250 (244)mV/cm, about 14-34 (24) hrs	9051-9060 (9056) MHz, 220-240 (238)mV/cm, about 4-12 (8) hrs	10171-10180 (10175)MHz, 250-270 (258)mV/cm, about 12-32 (22) hrs	11896-11905 (11898)MHz, 260-280 (265)mV/cm, about 4-12 (8) hrs	12786-12795 (12792) MHz, 280-300 (283)mV/cm, about 14-34 (24) hrs
Uterine Cancer	10161-10170 (10162)MHz, 220-240 (235)mV/cm, about 14-34 (24) hrs	11521-11530 (11526) MHz, 210-230 (226)mV/cm, about 4-12 (8) hrs	12131-12140 (12132)MHz, 255-275 (266)mV/cm, about 14-34 (24) hrs	12451-12460 (12458)MHz, 260-280 (265)mV/cm, about 4-12 (8) hrs	12696-12705 (12698) MHz, 270-290 (275)mV/cm, about 14-34 (24) hrs
Kidney Cancer	8036-8045 (8042) MHz, 210-230 (223)mV/cm, about 16-36	11061-11070 (11065) MHz, 205-225 (212)mV/cm, about 4-12 (8)	12081-12090 (12087)MHz, 235-255 (245)mV/cm, about 12-32	12361-12370 (12362)MHz, 230-250 (234)mV/cm, about 4-12	12861-12870 (12865) MHz, 260-280 (264)mV/cm, about 16-36

	(26) hrs	hrs	(22) hrs	(8) hrs	(26) hrs
Bladder Cancer	7891-7900 (7895) MHz, 230-250 (246)mV/cm, about 14-34 (24) hrs	10181-10190 (10184) MHz, 220-240 (236)mV/cm, about 4-12 (8) hrs	12146-12155 (12149)MHz, 250-270 (259)mV/cm, about 14-34 (24) hrs	12661-12670 (12665)MHz, 240-270 (246)mV/cm, about 4-12 (8) hrs	12891-12900 (12897) MHz, 280-300 (283)mV/cm, about 14-34 (24) hrs
Brain Cancer	8006-8015 (8011) MHz, 300-320 (315)mV/cm, about 14-34 (24) hrs	9141-9150 (9147) MHz, 300-320 (312)mV/cm, about 4-12 (8) hrs	9936-9945 (9941)MHz, 345-365 (356)mV/cm, about 14-34 (24) hrs	11331-11340 (11335)MHz, 330-350 (332)mV/cm, about 4-12 (8) hrs	12031-12040 (12032) MHz, 370-390 (375)mV/cm, about 14-34 (24) hrs
Lym- phoma	7972-7983 (7977) MHz, 250-300 (282)mV/cm, about 10-30 (20)hrs	7993-8003 (7998) MHz, 250-300 (277)mV/cm, about 10-30 (20)hrs	10024-10034 (10029)MHz, 370-400 (387)mV/cm, about 12-32 (22)hrs	12832-12842 (12837)MHz, 420-450 (442)mV/cm, about 12-32 (22)hrs	12857-12867 (12862) MHz, 310-350 (325)mV/cm, about 6-18 (12)hrs
Leu- kemia	9971-9980 (9978) MHz, 200-220 (217)mV/cm, about 14-34 (24) hrs	11481-11490 (11484) MHz, 190-210 (207)mV/cm, about 4-12 (8) hrs	12191-12200 (12196)MHz, 230-250 (238)mV/cm, about 14-34 (24) hrs	12561-12570 (12565)MHz, 240-270 (244)mV/cm, about 4-12 (8) hrs	12611-12620 (12612) MHz, 260-280 (262)mV/cm, about 14-34 (24) hrs

Condition of Yeast Cells

The following electromagnetic (EM) fields can be used to condition

- 5 the activated yeast cells in the biological compositions of the invention. The activated yeast cells were conditioned by culturing in a liquid medium comprising wild hawthorn juice and gastric juice of a mammal, and subjected to at least one of the two EM fields as shown below in Table 5 (numbers shown in parentheses are for the preferred embodiments) by following the general procedures set forth in the Detailed
- 10 Description of the Invention.

Table 5

Type of Cancer	1 st EM Field	2 nd EM Field
Lung Cancer	12716-12726 (12721)Hz, 380-420 (386) mV/cm, about 30-50 (40)hrs	12750-12760 (12755)MHz, 280-320 (295) mV/cm, about 12-32 (22)hrs
Nasopharyngeal Cancer	12441-12450 (12446)Hz, 260-300 (275) mV/cm, about 6-18 (12) hrs	12751-12760 (12757)MHz, 290-310 (303) mV/cm, about 26-46 (36) hrs
Esophageal	11241-11250 (11248)Hz,	12651-12660 (12652)MHz,

Cancer	280-320 (312) mV/cm, about 6-18 (12) hrs	320-340 (333) mV/cm, about 27-47 (37) hrs
Stomach Cancer	11221-11230 (11224)Hz, 300-320 (315) mV/cm, about 6-18 (12) hrs	12166-12175 (12171)MHz, 340-360 (346) mV/cm, about 28-48 (38) hrs
Colorectal Cancer	11141-11150 (11148)Hz, 230-250 (242) mV/cm, about 6-18 (12) hrs	12771-12780 (12778)MHz, 290-310 (293) mV/cm, about 26-46 (36) hrs
Pancreatic Cancer	12461-12470 (12466)Hz, 300-320 (306) mV/cm, about 6-18 (12) hrs	12761-12770 (12764)MHz, 300-330 (323) mV/cm, about 22-42 (32) hrs
Liver Cancer	12121-12130 (12126)Hz, 420-440 (422) mV/cm, about 6-18 (12) hrs	12851-12860 (12854)MHz, 400-450 (437) mV/cm, about 30-50 (40) hrs
Testicular Cancer	12061-12070 (12066)Hz, 320-340 (326) mV/cm, about 5-15 (10) hrs	12731-12740 (12737)MHz, 340-360 (346) mV/cm, about 24-44 (34) hrs
Prostate Cancer	12536-12545 (12541)Hz, 260-280 (265) mV/cm, about 5-15 (10) hrs	12656-12665 (12661)MHz, 280-300 (298) mV/cm, about 22-42 (32) hrs
Ovarian Cancer	12341-12350 (12346)Hz, 280-300 (284) mV/cm, about 4-12 (8) hrs	12781-12790 (12786)MHz, 290-310 (306) mV/cm, about 32-52 (42) hrs
Breast Cancer	12351-12360 (12355)Hz, 280-300 (286) mV/cm, about 6-18 (12) hrs	12791-12800 (12798)MHz, 280-300 (298) mV/cm, about 28-48 (38) hrs
Cervical Cancer	11896-11905 (11898)Hz, 280-300 (288) mV/cm, about 6-18 (12) hrs	12786-12795 (12792)MHz, 290-310 (307) mV/cm, about 24-44 (34) hrs
Uterine Cancer	12451-12460 (12458)Hz, 280-300 (284) mV/cm, about 6-18 (12) hrs	12696-12705 (12698)MHz, 280-300 (297) mV/cm, about 24-44 (34) hrs
Kidney Cancer	12361-12370 (12362)Hz, 245-265 (250) mV/cm, about 6-18 (12) hrs	12861-12870 (12865)MHz, 265-285 (282) mV/cm, about 32-52 (42) hrs
Bladder Cancer	12661-12670 (12665)Hz, 255-275 (256) mV/cm, about 6-18 (12) hrs	12891-12900 (12897)MHz, 270-300 (293) mV/cm, about 24-44 (34) hrs
Brain Cancer	11331-11340 (11335)Hz, 300-320 (306) mV/cm, about 6-18 (12) hrs	12031-12040 (12032)MHz, 305-325 (322) mV/cm, about 24-44 (34) hrs
Lymphoma	12832-12842 (12837)Hz, 380-400 (391) mV/cm, about 29-49 (39)hrs	12857-12867 (12862)MHz, 300-350 (315) mV/cm, about 10-30 (20)hrs
Leukemia	12561-12570 (12565)Hz, 260-290 (265) mV/cm, about 6-18 (12) hrs	12611-12620 (12612)MHz, 260-280 (278) mV/cm, about 29-49 (39) hrs

Large-scale Manufacturing of Yeast Cells

The following EM fields (numbers shown in parentheses are for the preferred embodiments) can be applied in accordance with the steps set forth in the Detailed Description of the Invention to mass produce the activated and conditioned yeast cells that were used to prepare the biological compositions that were

5 administered to the animals used in the respective human cancer type.

Table 6

Cancer Type	First Container (5)		Second Container (6)		Third Container (7)	
	1 st EM Field	2 nd EM Field	1 st EM Field	2 nd EM Field	1 st EM Field	2 nd EM Field
Lung Cancer	12,716-12,726 (12,721) MHz, 210-450 (422) mV/cm, about 5-25 (15) hrs	12,750-12,760 (12,755) MHz, 145-400 (388) mV/cm, about 6-18 (12) hrs	12,716-12,726 (12,721) MHz, 320-350 (341) mV/cm, about 6-18 (12) hrs	12,750-12,760 (12,755) MHz, 220-250 (231) mV/cm, about 5-15 (10) hrs	12,716-12,726 (12,721) MHz, 210-250 (234) mV/cm, about 12-32 (22) hrs	12,750-12,760 (12,755) MHz, 145-165 (154) mV/cm, about 6-18 (12) hrs
Nasopharyngeal Cancer	12,441-12,450 (12,446) MHz, 310-330 (312) mV/cm, about 5-15 (10) hrs	12,751-12,760 (12,757) MHz, 310-330 (327) mV/cm, about 6-18 (12) hrs	12,441-12,450 (12,446) MHz, 240-255 (250) mV/cm, about 5-15 (10) hrs	12,751-12,760 (12,757) MHz, 330-350 (343) mV/cm, about 6-18 (12) hrs	12,441-12,450 (12,446) MHz, 215-235 (220) mV/cm, about 6-18 (12) hrs	12,751-12,760 (12,757) MHz, 240-260 (251) mV/cm, about 14-34 (24) hrs
Esophageal Cancer	11,241-11,250 (11,248) MHz, 310-330 (322) mV/cm, about 4-12 (8) hrs	12,651-12,660 (12,652) MHz, 330-360 (356) V/cm, about 5-15 (10) hrs	11,241-11,250 (11,248) MHz, 320-340 (332) mV/cm, about 4-12 (8) hrs	12,651-12,660 (12,652) MHz, 340-360 (357) mV/cm, about 5-15 (10) hrs	11,241 to 11,250 (11,248) MHz, 210-230 (211) mV/cm, about 6-18 (12) hrs	12,651-12,660 (12,652) MHz, 230-250 (238) mV/cm, about 14-34 (24) hrs
Stomach Cancer	11,221-11,230 (11,224) MHz, 310-330 (322) mV/cm, about 4-12 (8) hrs	12,166-12,175 (12,171) MHz, 360-380 (377) mV/cm, about 6-18 (12) hrs	11,221-11,230 (11,224) MHz, 340-360 (346) mV/cm, about 4-12 (8) hrs	12,166-12,175 (12,171) MHz, 380-410 (404) mV/cm, about 4-24 (14) hrs	11,221-11,230 (11,224) MHz, 215 to 235 (222) mV/cm, about 6-18 (12) hrs	12,166-12,175 (12,171) MHz, 230-250 (242) mV/cm, about 12-32 (22) hrs
Colorectal	11,141-	12,771-	11,141-	12,771-	11,141-	12,771-

Cancer	11,150 (11,148) MHz, 280-300 (286) mV/cm, for about 4-12 (8) hrs	12,780 (12,778) MHz, 310-330 (322) mV/cm, about 5-15 (10) hrs	11,150 (11,148) MHz, 280-300 (292) mV/cm, about 4-12 (8) hrs	12,780 (12,778) MHz, 330-350 (332) mV/cm, about 6-18 (12) hrs	11,150 (11,148) MHz, 180-200 (188) mV/cm, about 6-18 (12) hrs	12,780 (12,778) MHz, 230-250 (236) mV/cm, about 14- 34 (24) hrs
Pancreatic Cancer	12,461- 12,470 (12,466) MHz, 310-330 (328) mV/cm, about 4-12 (8) hrs	12,761- 12,770 (12,764) MHz, 330-350 (338) mV/cm, about 6-18 (12) hrs	12,461- 12,470 (12,466) MHz, 330-340 (334) mV/cm, about 4-12 (8) hrs	12,761- 12,770 (12,764) MHz, 350-370 (362) mV/cm, about 6-18 (12) hrs	12,461- 12,470 (12,466) MHz, 220-240 (238) mV/cm, about 6-18 (12) hrs	12,761- 12,770 (12,764) MHz, 250-270 (263) mV/cm, about 14- 34 (24) hrs
Liver Cancer	12,121- 12,130 (12,126) MHz, 400-420 (412) mV/cm, for about 5-15 (10) hrs	12,851- 12,860 (12,854) MHz, 420-450 (436) mV/cm, about 4-24 (14) hrs	12,121- 12,130 (12,126) MHz, 300-320 (308) mV/cm, about 5-15 (10) hrs	12,851- 12,860 (12,854) MHz, 320-350 (325) mV/cm, about 6-18 (12) hrs	12,121 to 12,130 (12,126) MHz, 210-230 (211) mV/cm, about 5-15 (10) hrs	12,851- 12,860 (12,854) MHz, 230-250 (235) mV/cm, about 14- 34 (24) hrs
Testicular Cancer	12,061- 12,070 (12,066) MHz, 320-340 (334) mV/cm, about 5-15 (10) hrs	12,731- 12,740 (12,737) MHz, 350-370 (367) mV/cm, about 6-18 (12) hrs	12,061- 12,070 (12,066) MHz, 330-350 (346) mV/cm, about 5-15 (10) hrs	12,731- 12,740 (12,737) MHz, 400-420 (412) mV/cm, about 6-18 (12) hrs	12,061- 12,070 (12,066) MHz, 340-360 (346) mV/cm, about 6-18 (12) hrs	12,731- 12,740 (12,737) MHz, 400-420 (417) mV/cm, about 14- 34 (24) hrs
Prostate Cancer	12,536- 12,545 (12,541) MHz, 250-270 (260) mV/cm, about 4-12 (8) hrs	12,656- 12,665 (12,661) MHz, 320-340 (322) mV/cm, about 6-18 (12) hrs	12,536- 12,545 (12,541) MHz, 260-280 (267) mV/cm, about 4-12 (8) hrs	12,656- 12,665 (12,661) MHz, 320-340 (338) mV/cm, about 6-18 (12) hrs	12,536- 12,545 (12,541) MHz, 170-190 (188) mV/cm, about 6-18 (12) hrs	12,656- 12,665 (12,661) MHz, 230-250 (245) mV/cm, about 14- 34 (24) hrs
Ovarian Cancer	12,341- 12,350 (12,346) MHz, 300-320 (311)	12,781- 12,790 (12,786) MHz, 320-340 (322)	12,341- 12,350 (12,346) MHz, 320-340 (323)	12,781- 12,790 (12,786) MHz, 330-350 (342)	12,341- 12,350 (12,346) MHz, 230-240 (236)	12,781- 12,790 (12,786) MHz, 220-240 (234)

	mV/cm, about 4-12 (8) hrs	mV/cm, about 6-18 (12) hrs	mV/cm, about 4-12 (8) hrs	mV/cm, about 6-18 (12) hrs	mV/cm, about 5-15 (10) hrs	mV/cm, about 12- 32 (22) hrs
Breast Cancer	12,351- 12,360 (12,355) MHz, 300-320 (312) mV/cm, about 4-12 (8) hrs	12,791- 12,800 (12,798) MHz, 310-330 (317) mV/cm, about 6-18 (12) hrs	12,351- 12,360 (12,355) MHz, 320-340 (322) mV/cm, about 4-12 (8) hrs	12,791- 12,800 (12,798) MHz, 330-350 (348) mV/cm, about 6-18 (12) hrs	12,351- 12,360 (12,355) MHz, 210-230 (211) mV/cm, about 6-18 (12) hrs	12,791- 12,800 (12,798) MHz, 210-230 (226) mV/cm, about 14- 34 (24) hrs
Cervical Cancer	11,896- 11,905 (11,898) MHz, 290-310 (305) mV/cm, about 4-12 (8) hrs	12,786- 12,795 (12,792) MHz, 320-340 (322) mV/cm, about 6-18 (12) hrs	11,896- 11,905 (11,898) MHz, 310-330 (315) mV/cm, about 4-12 (8) hrs	12,786- 12,795 (12,792) MHz, 320-340 (337) mV/cm, about 6-18 (12) hrs	11,896- 11,905 (11,898) MHz, 210-230 (228) mV/cm, about 6-18 (12) hrs	12,786- 12,795 (12,792) MHz, 240-260 (255) mV/cm, about 14- 34 (24) hrs
Uterine Cancer	12,451- 12,460 (12,458) MHz, 300-320 (316) mV/cm, about 4-12 (8) hrs	12,696- 12,705 (12,698) MHz, 320-340 (327) mV/cm, about 6-18 (12) hrs	12,451- 12,460 (12,458) MHz, 320-340 (328) mV/cm, about 4-12 (8) hrs	12,696- 12,705 (12,698) MHz, 330-350 (346) mV/cm, about 6-18 (12) hrs	12,451- 12,460 (12,458) MHz, 250-270 (262) mV/cm, about 6-18 (12) hrs	12,696- 12,705 (12,698) MHz, 250-270 (262) mV/cm, about 14- 34 (24) hrs
Kidney Cancer	12,361- 12,370 (12,362) MHz, 245-265 (262) mV/cm, about 4-12 (8) hrs	12,861- 12,870 (12,865) MHz, 300-320 (302) mV/cm, about 6-18 (12) hrs	12,361- 12,370 (12,362) MHz, 265-285 (270) mV/cm, about 4-12 (8) hrs	12,861- 12,870 (12,865) MHz, 305-325 (321) mV/cm, about 6-18 (12) hrs	12,361- 12,370 (12,362) MHz, 195-215 (212) mV/cm, about 6-18 (12) hrs	12,861- 12,870 (12,865) MHz, 245-265 (262) mV/cm, about 14- 34 (24) hrs
Bladder Cancer	12,661- 12,670 (12,665) MHz, 240-260 (256) mV/cm, about 4-12 (8) hrs	12,891- 12,900 (12,897) MHz, 320-340 (322) mV/cm, about 6-18 (12) hrs	12,661- 12,670 (12,665) MHz, 260-280 (266) mV/cm, about 4-12 (8) hrs	12,891- 12,900 (12,897) MHz, 320-340 (332) mV/cm, about 6-18 (12) hrs	12,661- 12,670 (12,665) MHz, 170-190 (188) mV/cm, about 6-18 (12) hrs	12,891- 12,900 (12,897) MHz, 230-250 (247) mV/cm, about 14- 34 (24) hrs
Brain Cancer	11,331- 11,340	12,031- 12,040	11,331- 11,340	12,031- 12,040	11,331- 11,340	12,031- 12,040

	(11,335) MHz, 300-320 (318) mV/cm, about 4-12 (8) hrs	(12,032) MHz, 350-370 (357) mV/cm, about 6-18 (12) hrs	(11,335) MHz, 330-350 (335) mV/cm, about 4-12 (8) hrs	(12,032) MHz, 390-410 (406) mV/cm, about 6-18 (12) hrs	(11,335) MHz, 335-335 (350) mV/cm, about 6-18 (12) hrs	(12,032) MHz, 390-420 (415) mV/cm, about 14- 34 (24) hrs
Lymphoma	12,832- 12,842 (12,837) MHz, 420-450 (442) mV/cm, about 6-18 (12) hrs	12,857- 12,867 (12,862) MHz, 340-380 (350) mV/cm, about 5-15 (10) hrs	12,832- 12,842 (12,837) MHz, 330-350 (341) mV/cm, about 4-24 (14) hrs	12,857- 12,867 (12,862) MHz, 210-240 (224) mV/cm, about 5-15 (10) hrs	12,832- 12,842 (12,837) MHz, 230-250 (244) mV/cm, about 12- 32 (22) hrs	12,857- 12,867 (12,862) MHz, 120-150 (137) mV/cm, about 6-18 (12) hrs
Leukemia	12,561- 12,570 (12,565) MHz, 255- 275 (270) mV/cm, about 4-12 (8) hrs	12,611- 12,620 (12,612) MHz, 290-310 (296) mV/cm, about 6-18 (12) hrs	12,561- 12,570 (12,565) MHz, 270-290 (277) mV/cm, about 4-12 (8) hrs	12,611- 12,620 (12,612) MHz, 290-320 (318) mV/cm, about 6-18 (12) hrs	12,561- 12,570 (12,565) MHz, 170-190 (184) mV/cm, about 6-18 (12) hrs	12,611- 12,620 (12,612) MHz, 250-270 (267) mV/cm, about 14- 34 (24) hrs

Lung Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a

5 subject suffering from lung cancer. As used herein, the term "lung cancer" includes but is not limited to squamous cell carcinoma, small cell carcinoma, adenocarcinoma, large cell carcinoma, carcinoid tumor, and mesothelioma. In a specific embodiment, the biological composition can ameliorate, reduce, manage, or eliminate the symptoms associated with lung cancer, such as cough, shortness of breath, wheezing,

10 chest pain, hemoptysis (bloody, coughed-up sputum), loss of appetite, weight loss, pneumonia (inflammation of the lungs), weakness, chills, swallowing difficulties, speech difficulties or changes (*e.g.*, hoarseness), finger/nail abnormalities (*e.g.*, "clubbing," or overgrowth of the fingertip tissue), skin paleness or bluish discoloration, muscle contractions or atrophy (shrinkage), joint pain or swelling,

15 facial swelling or paralysis, eyelid drooping, bone pain/tenderness, and breast development in men.

According to the invention, the biological composition useful for treatment of lung cancer comprises activated and conditioned *Saccharomyces cerevisiae* Hansen strain IFFI1345 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing
5 said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 8,051 MHz and a field strength of 293 mV/cm;
 - (b) culturing the yeast cells in a first liquid medium in a second
10 electromagnetic field having a frequency at 8,082 MHz and a field strength of 272 mV/cm;
 - (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 9,912 MHz and a field strength of 354 mV/cm;
 - (d) culturing the yeast cells in a first liquid medium in a fourth
15 electromagnetic field having a frequency at 12,721 MHz and a field strength of 398 mV/cm; and
 - (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,755 MHz and a field
20 strength of 302 mV/cm;
- and after the last of the first five steps, the following steps in any order:
- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,721 MHz and a field strength of 386 mV/cm; and
 - (g) culturing the yeast cells in a second liquid medium in a seventh
25 electromagnetic field having a frequency at 12,755 MHz and a field strength of 295 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μ g of vitamin B₂, 30 μ g of vitamin B₆, 30 μ g of vitamin B₁₂, 0.20 g of
30 KH₂PO₄, 0.22 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.30 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,721 MHz and a field strength in the range of 210 to 450 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 422 mV/cm, 341 mV/cm, and 234 mV/cm; and
- (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,755 MHz and a field strength in the range of 145 to 400 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 388 mV/cm, 231 mV/cm, and 154 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Nasopharyngeal Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from nasopharyngeal cancer. As used herein, the term “nasopharyngeal cancer” includes but is not limited to squamous cell carcinoma, nonkeratinizing carcinomas, undifferentiated carcinomas and keratinizing carcinoma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms of the cancer, improving the probability of survival of the subject with the cancer, prolonging the life expectancy of the subject, improving the quality of life of the subject, and/or reducing the probability of relapse after a successful course of treatment (*e.g.*, surgery, chemotherapy or radiation). The symptoms associated with nasopharyngeal cancer include neck mass, hearing loss, ipsilateral serous otitis, hearing loss, nasal obstruction, frank epistaxis, purulent or bloody rhinorrhea, and facial neuropathy or facial nerve palsies.

According to the invention, the biological composition useful for treatment of nasopharyngeal cancer comprises activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.116 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 9,963 MHz and a field strength of 246 mV/cm;
- (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 10,956 MHz and a field strength of 250 mV/cm;
- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,098 MHz and a field strength of 263 mV/cm;
- (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,446 MHz and a field strength of 244 mV/cm; and
- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,757 MHz and a field strength of 276 mV/cm;
- and after the last of the first five steps, the following steps in any order:
- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,446 MHz and a field strength of 275 mV/cm; and
- (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,757 MHz and a field strength of 303 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μ g of vitamin B₆, 40 μ g of vitamin B₁₂, 70 μ g of vitamin H, 25 ml of fetal calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,446 MHz and a field strength in the range of 215 to

330 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 312 mV/cm, 250 mV/cm, and 220 mV/cm; and

- (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,757 MHz and a field strength in the range of 240 to 350 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 327 mV/cm, 343 mV/cm, and 251 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Esophageal Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from esophageal cancer. As used herein, the term "esophageal cancer" includes but is not limited to squamous cell carcinoma, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, carcinosarcoma, pseudosarcoma, sarcomas, melanoma, plasmacytoma, verrucous carcinoma and oat cell carcinoma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with esophageal cancer include dysphagia, difficulty swallowing solids or liquids, regurgitation of food, heartburn, vomiting blood and chest pain unrelated to eating.

According to the invention, the biological composition useful for treatment of esophageal cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain AS2.375 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,846 MHz and a field strength of 272 mV/cm;
- (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 8,926 MHz and a field strength of 262 mV/cm;
- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 10,192 MHz and a field strength of 293 mV/cm;

- (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 11,248 MHz and a field strength of 280 mV/cm; and
- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,652 MHz and a field strength of 317 mV/cm;
- 5 and after the last of the first five steps, the following steps in any order:
- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 11,248 MHz and a field strength of 312 mV/cm; and
- 10 (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,652 MHz and a field strength of 333 mV/cm.

Preferably, the first liquid medium comprises 10 g of sucrose or glucose, 10 g of soluble starch, 50 μ g of vitamin B₂, 50 μ g of vitamin B₆, 20 μ g of vitamin B₁₂, 35 ml of fetal calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 11,248 MHz and a field strength in the range of 210 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 332 mV/cm, and 211 mV/cm; and
- 25 (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,652 MHz and a field strength in the range of 230 to 360 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 356 mV/cm, 357 mV/cm, and 238 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Stomach Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from stomach cancer. As used herein, the term "stomach cancer" includes but is not limited to malignant cancer such as adenocarcinoma, papillary adenocarcinoma, tubular adenocarcinoma, mucinous adenocarcinoma, signet ring cell carcinoma, adenosquamous carcinoma, carcinoid tumor, mixed carcinoid-adenocarcinoma, small cell carcinoma (M80413), and undifferentiated carcinoma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with stomach cancer include loss of appetite, difficulty in swallowing, vague fullness, vomiting blood, abdominal pain, belching, breath odor, excessive gas and flatus, heartburn, weight loss, and a decline in general health.

According to the invention, the biological composition useful for treatment of stomach cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain AS2.14 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,987 MHz and a field strength of 286 mV/cm;
- (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 9,137 MHz and a field strength of 281 mV/cm;
- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 10,178 MHz and a field strength of 312 mV/cm;
- (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 11,224 MHz and a field strength of 310 mV/cm; and
- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,171 MHz and a field strength of 332 mV/cm;

and after the last of the first five steps, the following steps in any order:

- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency 11,224 MHz and a field strength of 315 mV/cm; and
- 5 (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency 12,171 MHz and a field strength of 346 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μ g of vitamin B₃, 30 μ g of vitamin B₆, 50 μ g of vitamin C, 25 ml of fetal calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of 10 CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

15 The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 11,224 MHz and a field strength in the range of 215 to 20 360 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 346 mV/cm, and 222 mV/cm; and
- (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,171 MHz and a field strength in the range of 230 to 25 410 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 377 mV/cm, 404 mV/cm, and 242 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

30 Colorectal Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from colorectal cancer. As used herein, the term "colorectal cancer" includes but is not limited to squamous cell (epidermoid) carcinomas, cloacogenic

(basaloid transitional cell) tumors, and adenocarcinomas. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with colorectal cancer include constipation, blood in the stool, unexplained anemia, abdominal pain and tenderness in the lower abdomen, intestinal
5 obstruction, weight loss with no known reason, stools narrower than usual, constant tiredness, and anal lump.

According to the invention, the biological composition useful for treatment of colorectal cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain AS2.1396 yeast cells cultured in a series of
10 alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,948 MHz and a field strength of 231 mV/cm;
- 15 (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 9,135 MHz and a field strength of 221 mV/cm;
- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 9,997 MHz and a field
20 strength of 254 mV/cm;
- (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 11,148 MHz and a field strength of 233 mV/cm; and
- (e) culturing the yeast cells in a first liquid medium in a fifth
25 electromagnetic field having a frequency at 12,778 MHz and a field strength of 266 mV/cm;

and after the last of the first five steps, the following steps in any order:

- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 11,148 MHz and a field
30 strength of 242 mV/cm; and
- (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,778 MHz and a field strength of 293 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 50 μ g of vitamin B₆, 50 μ g of vitamin B₁₂, 30 ml of fetal calf serum, 60 μ g of vitamin A, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved
5 water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- 10 (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 11,148 MHz and a field strength in the range of 180 to 300 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 286 mV/cm, 292 mV/cm, and 188 mV/cm; and
- 15 (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,778 MHz and a field strength in the range of 230 to 350 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 332 mV/cm, and 236 mV/cm.

20 Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Pancreatic Cancer

In one preferred embodiment, the invention provides a method for
25 preparing a biological composition useful for producing a healthful benefit in a subject suffering from pancreatic cancer. As used herein, the term "pancreatic cancer" includes but is not limited to adenocarcinomas, acinar cell carcinoma, cystadenocarcinoma (mucinous), adenosquamous carcinoma, solid microglandular carcinoma, carcinoid, sarcoma, and malignant lymphoma. In a specific embodiment,
30 the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with pancreatic cancer include abdominal pain, unexpected weight loss, nausea, loss of appetite, weight loss, digestive problems, jaundice, or yellowing of the skin, restlessness, loss of energy, irritability, sweating, tremor, drowsiness and severe confusion.

According to the invention, the biological composition useful for treatment of pancreatic cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain IFFI 1413 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for

5 preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,967 MHz and a field strength of 264 mV/cm;
- 10 (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 10,188 MHz and a field strength of 266 mV/cm;
- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,281 MHz and a field strength of 279 mV/cm;
- 15 (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,466 MHz and a field strength of 286 mV/cm; and
- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,764 MHz and a field strength of 306 mV/cm;
- 20

and after the last of the first five steps, the following steps in any order:

- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,466 MHz and a field strength of 306 mV/cm; and
- 25 (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,764 MHz and a field strength of 323 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 50 μ g of vitamin B₃, 30 μ g of vitamin B₁₂, 20 μ g of vitamin H, 40 ml of fetal calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

30

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,466 MHz and a field strength in the range of 220 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 328 mV/cm, 334 mV/cm, and 238 mV/cm; and
- (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,764 MHz and a field strength in the range of 250 to 370 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 338 mV/cm, 362 mV/cm, and 263 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Liver Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from liver cancer. As used herein, the term "liver cancer" includes but is not limited to hepatocellular carcinoma, cholangiocarcinoma, mixed hepatocellular cholangiocarcinoma, angiosarcoma, fibrolamellar, cystadenoma, and epithelioid hemangioendothelioma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with liver cancer include general malaise as well as pain and tenderness, unexplained weight loss, persistent lack of appetite, fever of unknown origin, limb weakness, sensory loss, persistent abdominal pain, immature feeling of fullness, swelling of the abdominal are with or without breathing difficulties, sudden jaundice, and liver enlargement or a mass that can be felt in the liver area.

According to the invention, the biological composition useful for treatment of liver cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain AS2.503 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,976 MHz and a field strength of 335 mV/cm;
- 5 (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 8,845 MHz and a field strength of 310 mV/cm;
- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 10,152 MHz and a field strength of 422 mV/cm;
- 10 (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,126 MHz and a field strength of 416 mV/cm; and
- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,854 MHz and a field strength of 445 mV/cm;
- 15 and after the last of the first five steps, the following steps in any order:
- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,126 MHz and a field strength of 422 mV/cm; and
- 20 (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,854 MHz and a field strength of 437 mV/cm.

Preferably, the first liquid medium comprises 30 g of sucrose or glucose, 40 μ g of vitamin B₆, 30 μ g of vitamin H, 40 μ g of vitamin A, 30 ml of bovine calf serum, 0.20 g of KH₂PO₄, 0.20 g of MgSO₄·7H₂O, 0.20 g of NaCl, 0.20 g of CaSO₄·2H₂O, 3.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

25

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

30 The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,126 MHz and a field strength in the range of 210 to

420 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 412 mV/cm, 308 mV/cm, and 211 mV/cm; and

- (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,854 MHz and a field strength in the range of 230 to 450 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 436 mV/cm, 325 mV/cm, and 235 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

10 **Testicular Cancer**

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from testicular cancer. As used herein, the term "testicular cancer" includes but is not limited to malignant cancer such as seminomas, nonseminomas, choriocarcinoma, embryonal carcinoma, immature teratoma, yolk sac tumors, Leydig and sertoli cell tumors, PNET, leiomyosarcoma, rhabdomyosarcoma, and mesothelioma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with testicular cancer include a painless lump, a hardening or a change (increase or decrease) in size of the testicle, a feeling of heaviness or a sudden collection of fluid in the scrotum, a dull ache in the lower abdomen or in the groin, or pain or discomfort in the scrotum or testicle.

According to the invention, the biological composition useful for treatment of testicular cancer comprises activated and conditioned are *Saccharomyces carlsbergensis* Hansen strain AS2.116 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 8,028 MHz and a field strength in the range of 327 mV/cm;
- (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 9,016 MHz and a field strength in the range of 326 mV/cm;

- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 9,941 MHz and a field strength in the range of 365 mV/cm;
- (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,066 MHz and a field strength in the range of 350 mV/cm; and
- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,737 MHz and a field strength in the range of 387 mV/cm;
- and after the last of the first five steps, the following steps in any order:
- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,066 MHz and a field strength in the range of 326 mV/cm; and
- (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,737 MHz and a field strength in the range of 346 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 60 μ g of vitamin B₁₂, 60 μ g of vitamin B₃, 60 μ g of vitamin H, 50 μ g of vitamin B₆, 30 ml of fetal calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,066 MHz and a field strength in the range of 320 to 360 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 334 mV/cm, 346 mV/cm, and 346 mV/cm; and
- (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,737 MHz and a field strength in the range of 350 to

420 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 367 mV/cm, 412 mV/cm, and 417 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

5 Prostate Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from prostate cancer. As used herein, the term "prostate cancer" includes but is not limited to adenocarcinomas. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with prostate cancer include frequent urination (especially at night) or an inability to urinate, trouble starting or holding back urine, pain during ejaculation or urination, a weak or interrupted urine flow, blood in the semen or in the urine, or frequent pain or stiffness in the lower back, hips, or upper thighs.

15 According to the invention, the biological composition useful for treatment of prostate cancer comprises activated and conditioned are *Saccharomyces carlsbergensis* Hansen strain AS2.440 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- 20 (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 10,164 MHz and a field strength of 239 mV/cm;
- (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 11,218 MHz and a field strength of 232 mV/cm;
- 25 (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,181 MHz and a field strength of 258 mV/cm;
- (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,541 MHz and a field strength of 251 mV/cm; and
- 30

- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,661 MHz and a field strength of 283 mV/cm;

and after the last of the first five steps, the following steps in any order:

- 5 (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,541 MHz and a field strength of 265 mV/cm; and
- (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,661 MHz and a field strength of 298 mV/cm.
- 10

Preferably, the first liquid medium comprises 10 g of sucrose or glucose, 10 g of mannitol, 80 μ g of vitamin B₆, 50 μ g of vitamin B₃, 60 μ g of vitamin H, 60 μ g of vitamin A, 30 ml of bovine calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

15

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- 20 (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,541 MHz and a field strength in the range of 170 to 280 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 260 mV/cm, 267 mV/cm, and 188 mV/cm ; and
- 25 (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,661 MHz and a field strength in the range of 230 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 338 mV/cm, and 245 mV/cm .

30 Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Ovarian Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from ovarian cancer. As used herein, the term "ovarian cancer" includes but is not limited to serous carcinoma and those arising from the celomic epithelium, specialized stroma, and germ cell layer or unfertilized ovum. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with ovarian cancer include abdominal pain, abdominal swelling, bloating or dyspepsia, pelvic pressure, weight gain or loss, abnormal menstrual cycles, increased abdominal girth, vaginal bleeding, excessive hair, and increased urinary frequency or urgency.

According to the invention, the biological composition useful for treatment of ovarian cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain AS2.502 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 10,088 MHz and a field strength of 243 mV/cm;
 - (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 11,214 MHz and a field strength of 228 mV/cm;
 - (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,142 MHz and a field strength of 257 mV/cm;
 - (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,346 MHz and a field strength of 216 mV/cm; and
 - (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,786 MHz and a field strength of 283 mV/cm;
- and after the last of the first five steps, the following steps in any order:
- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency 12,346 MHz and a field strength of 284 mV/cm; and

- (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,786 MHz and a field strength of 306 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or
5 glucose, 40 μ g of vitamin B₁₂, 30 μ g of vitamin B₆, 50 μ g of vitamin A, 40 μ g of vitamin C, 35 ml of bovine calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice
10 and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a
15 frequency at 12,346 MHz and a field strength in the range of 230 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 311 mV/cm, 323 mV/cm, and 236 mV/cm; and

- (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a
20 frequency 12,786 MHz and a field strength in the range of 220 to 350 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 342 mV/cm, and 234 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

25 **Breast Cancer**

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from breast cancer. As used herein, the term "breast cancer" includes but is not limited to malignant epithelial cancer such as adenocarcinomas (ductal
30 carcinomas and lobular carcinomas), squamous cell carcinoma, nonkeratinizing carcinomas, undifferentiated carcinomas, and keratinizing carcinoma, ductal carcinoma in situ (DCIS), infiltrating (or invasive) ductal carcinoma (IDC), infiltrating (or invasive) lobular carcinoma (ILC), inflammatory breast cancer, in situ,

lobular carcinoma in situ (LCIS), medullary carcinoma, mucinous carcinoma, phyllodes tumor, tubular carcinoma, and Paget's disease of the nipple. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with breast cancer include breast lump(s), swelling
5 of the skin of the breast, discharge from the nipple, enlargement of the lymph glands of the armpit, nipple erosion or ulceration, diffuse erythema of the breast, and axillary adenopathy.

According to the invention, the biological composition useful for treatment of breast cancer comprises activated and conditioned *Saccharomyces*
10 *carlsbergensis* Hansen strain AS2.441 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first
15 electromagnetic field having a frequency at 8,066 MHz and a field strength of 236 mV/cm ;
 - (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 10,124 MHz and a field strength of 226 mV/cm;
 - (c) culturing the yeast cells in a first liquid medium in a third
20 electromagnetic field having a frequency at 12,246 MHz and a field strength of 264 mV/cm;
 - (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,355 MHz and a field strength of 275 mV/cm; and
 - (e) culturing the yeast cells in a first liquid medium in a fifth
25 electromagnetic field having a frequency at 12,798 MHz and a field strength of 277 mV/cm;
- and after the last of the first five steps, the following steps in any order:
- (f) culturing the yeast cells in a second liquid medium in a sixth
30 electromagnetic field having a frequency at 12,355 MHz and a field strength of 286 mV/cm; and
 - (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,798 MHz and a field strength of 298 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 30 μ g of vitamin B₁₂, 50 μ g of vitamin B₃, 40 μ g of vitamin H, 40 μ g of vitamin C, 35 ml of bovine calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and
5 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- 10 (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,355 MHz and a field strength in the range of 210 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 312 mV/cm, 322 mV/cm, and 211 mV/cm; and
- 15 (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,798 MHz and a field strength in the range of 210 to 350 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 317 mV/cm, 348 mV/cm, and 226 mV/cm.

20 Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Cervical Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a
25 subject suffering from cervical cancer. As used herein, the term "cervical cancer" includes but is not limited to adenocarcinomas (ductal carcinomas and lobular carcinomas), squamous cell carcinoma, nonkeratinizing carcinomas, undifferentiated carcinomas, and keratinizing carcinoma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated
30 with cervical cancer include abnormal bleeding, such as between periods or after intercourse, persistent vaginal discharge, which may be pale, watery, pink, brown, blood streaked, or dark and foul-smelling, and discomfort during intercourse.

According to the invention, the biological composition useful for treatment of cervical cancer comprises activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.444 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing

5 said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,825 MHz and a field strength of 244 mV/cm;
- 10 (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 9,056 MHz and a field strength of 238 mV/cm;
- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 10,175 MHz and a field strength of 258 mV/cm;
- 15 (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 11,898 MHz and a field strength of 265 mV/cm; and
- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,792 MHz and a field strength of 283 mV/cm;
- 20

and after the last of the first five steps, the following steps in any order:

- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency 11,898 MHz and a field strength of 288 mV/cm; and
- 25 (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,792 MHz and a field strength of 307 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μ g of vitamin B₁₂, 40 μ g of vitamin B₃, 50 μ g of vitamin H, 50 μ g of vitamin B₆, 45 ml of fetal calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 11,898 MHz and a field strength in the range of 210 to 330 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 305 mV/cm, 315 mV/cm, and 228 mV/cm; and
- (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,792 MHz and a field strength in the range of 240 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 337 mV/cm, and 255 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

15 **Uterine Cancer**

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from uterine cancer. As used herein, the term "uterine cancer" includes but is not limited to squamous cell carcinoma, endometrioid carcinoma, adenoacanthoma, adenosquamous carcinoma, papillary serous carcinomas, and clear cell adenocarcinomas. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with uterine cancer include abnormal vaginal and/or uterine spotting or bleeding, such as between periods or after intercourse, white or clear vaginal discharge, difficult or painful urination, discomfort during intercourse, pain or cramping in the pelvic area, and discomfort during intercourse .

According to the invention, the biological composition useful for treatment of uterine cancer comprises activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.605 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 10,162 MHz and a field strength of 235 mV/cm;
- 5 (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 11,526 MHz and a field strength of 226 mV/cm;
- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,132 MHz and a field strength of 266 mV/cm;
- 10 (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,458 MHz and a field strength of 265 mV/cm; and
- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,698 MHz and a field strength of 275 mV/cm;
- 15 and after the last of the first five steps, the following steps in any order:
- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,458 MHz and a field strength of 284 mV/cm; and
- 20 (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,698 MHz and a field strength of 297 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 60 μ g of vitamin B₁₂, 60 μ g of vitamin B₃, 60 μ g of vitamin H, 50 μ g of vitamin B₆, 30 ml of fetal calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

25

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

30 The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,458 MHz and a field strength in the range of 250 to

340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 316 mV/cm, 328 mV/cm, and 262 mV/cm; and

- (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,698 MHz and a field strength in the range of 250 to 350 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 327 mV/cm, 346 mV/cm, and 262 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

10 **Kidney Cancer**

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from kidney cancer. As used herein, the term "kidney cancer" includes but is not limited to adenocarcinomas, hypernephroma, renal cell carcinoma, clear cell cancer, and Grawitz's tumor. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with kidney cancer include blood in the urine (hematuria), persistent pain in the area between the ribs and the hip not associated with an injury, a mass in the area of the kidneys, high blood pressure, rapid and unexplained weight loss, persistent feeling of fatigue, fever not caused by a cold or flu, swelling of the legs and ankles, anemia, and intermittent fever.

According to the invention, the biological composition useful for treatment of kidney cancer comprises activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.189 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 8,042 MHz and a field strength of 223 mV/cm;
- (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 11,065 MHz and a field strength of 212 mV/cm;

- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,087 MHz and a field strength of 245 mV/cm;
- (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,362 MHz and a field strength of 234 mV/cm; and
- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,865 MHz and a field strength of 264 mV/cm;
- and after the last of the first five steps, the following steps in any order:
- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,362 MHz and a field strength of 250 mV/cm; and
- (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,865 MHz and a field strength of 282 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μ g of vitamin B₃, 30 μ g of vitamin B₆, 60 μ g of vitamin A, 20 μ g of vitamin H, 50 μ g of vitamin C, 30 ml of fetal calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 980 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,362 MHz and a field strength in the range of 195 to 285 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 262 mV/cm, 270 mV/cm, and 212 mV/cm; and
- (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,865 MHz and a field strength in the range of 245 to 325

mV/cm, preferably at three fields strengths, *e.g.*, in the order of 302 mV/cm, 321 mV/cm, and 262 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

5 **Bladder Cancer**

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from bladder cancer. As used herein, the term "bladder cancer" includes but is not limited to urothelial carcinoma, transitional cell carcinoma, 10 squamous cell carcinoma, adenocarcinoma, papillomas, flat urothelial carcinomas, and rhabdomyosarcoma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with bladder cancer include microscopic (visible only under a microscope) or gross (visible to the naked eye) hematuria, or blood in the urine, frequent urination, urinary urgency, 15 urinary frequency, painful urinary (dysuria), urinary incontinence, bone pain or tenderness, abdominal pain, anemia, weight loss, and lethargy.

According to the invention, the biological composition useful for treatment of bladder cancer comprises activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.4 yeast cells cultured in a series of alternating 20 electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,895 MHz and a field strength of 246 mV/cm;
- 25 (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 10,184 MHz and a field strength of 236 mV/cm;
- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,149 MHz and a field strength of 259 mV/cm;
- 30 (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,665 MHz and a field strength of 246 mV/cm; and

- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,897 MHz and a field strength of 283 mV/cm;

and after the last of the first five steps, the following steps in any order:

- 5 (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,665 MHz and a field strength of 256 mV/cm; and
- (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,897 MHz and a field strength of 293 mV/cm.

10 Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 50 μ g of vitamin B₁₂, 30 μ g of vitamin B₆, 20 μ g of vitamin H, 50 μ g of vitamin A, 30 ml of bovine calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and

15 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- 20 (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,665 MHz and a field strength in the range of 170 to 280 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 256 mV/cm, 266 mV/cm, and 188 mV/cm; and
- 25 (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,897 MHz and a field strength in the range of 230 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 332 mV/cm, and 247 mV/cm.

30 Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Brain Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from brain cancer. As used herein, the term "brain cancer" includes but is not limited to astrocytoma, meningioma, colloid cyst, ependymoma, metastatic tumors, choroid plexus papilloma, subependymoma, astrocytoma, glioblastoma, lipoma, oligodendroglioma, sarcoma, germ cell tumors, pineal cell tumors, chordoma, pituitary adenoma, craniopharyngioma, chordoma, acoustic schwannoma, glomus jugulare tumor, medulloblastoma, hemangioblastoma, glioglastoma multiforme, neurinomas, cerebellar astrocytoma, and brainstem glioma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with brain cancer in adults include recent onset or persistent headache, vomiting, personality and behavior changes, emotional instability, intellectual decline (e.g., confusion, loss of memory, impaired calculating abilities, and impaired judgment), seizures, reduced level of consciousness, neurologic changes (e.g., vision changes, hearing loss, decreased sensation of a body area, weakness of a body area, speech difficulties, and decreased coordination), fever, weakness, general ill feeling, positive Babinski's reflex, and decerebrate or decorticate posture. Common symptoms associated with brain cancer in infants include bulging fontanelles, separated sutures, opisthotonos, increased head circumference, no red reflex in the eye. Additional symptoms include tongue problems, difficulty swallowing, impaired smell, obesity, uncontrollable movement, absent menstruation, hiccups, hand tremor, facial paralysis, different pupil sizes, eyelid drooping, and breathing problem.

According to the invention, the biological composition useful for treatment of brain cancer comprises activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.501 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 8,011 MHz and a field strength of 315 mV/cm;
- (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 9,147 MHz and a field strength of 312 mV/cm;

- (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 9,941 MHz and a field strength of 356 mV/cm;
- (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 11,335 MHz and a field strength of 332 mV/cm; and
- (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,032 MHz and a field strength of 375 mV/cm;

and after the last of the first five steps, the following steps in any order:

- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 11,335 MHz and a field strength of 306 mV/cm; and
- (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,032 MHz and a field strength of 322 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 60 μ g of vitamin B₁₂, 60 μ g of vitamin B₃, 60 μ g of vitamin H, 50 μ g of vitamin B₆, 30 ml of fetal calf serum, 0.20 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 11,335 MHz and a field strength in the range of 300 to 355 mV/cm, preferably at three fields strengths, e.g., in the order of 318 mV/cm, 335 mV/cm, and 350 mV/cm; and
- (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,032 MHz and a field strength in the range of 350 to 420

mV/cm, preferably at three fields strengths, *e.g.*, in the order of 357 mV/cm, 406 mV/cm, and 415 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

5 **Lymphoma Cancer**

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from lymphoma cancer. As used herein, the term "lymphoma" includes but is not limited to Hodgkin's disease and non-Hodgkin's disease. For a detailed description of each type of lymphoma, see Rosenberg SA, Kaplan HS, eds. 10 "Malignant Lymphomas: Etiology, Immunology, Pathology, Treatment" New York, NY, Academic Press 1982, which is incorporated herein by reference in its entirety. Classic Hodgkin's disease is divided into four subtypes: (1) nodular sclerosis Hodgkin's disease (NSHD); (2) mixed cellularity Hodgkin's disease (MCHD); (3) 15 lymphocyte depletion Hodgkin's disease (LDHD); and (4) lymphocyte-rich classic Hodgkin's disease (cLRHD). Non-Hodgkin's lymphoma includes but is not limited to (1) slow-growing lymphoma and lymphoid leukemia (*e.g.*, chronic lymphocytic leukemia, small lymphocytic leukemia, lymphoplasmacytoid lymphoma, follicle center lymphoma, follicular small cleaved cell, follicular mixed cell, marginal zone B- 20 cell lymphoma, hairy cell leukemia, plasmacytoma, myeloma, large granular lymphocyte leukemia, mycosis fungoides, sézary syndrome); (2) moderately aggressive lymphomas and lymphoid leukemia (*e.g.*, prolymphocytic leukemia, mantle cell lymphoma, follicle center lymphoma, follicular small cleaved cell, follicle center lymphoma, chronic lymphocytic leukemia/prolymphocytic leukemia, 25 angiocentric lymphoma, angioimmunoblastic lymphoma); (3) aggressive lymphomas (*e.g.*, large B-cell lymphoma, peripheral T-cell lymphomas, intestinal T-cell lymphoma, anaplastic large cell lymphoma); and (4) highly aggressive lymphomas and lymphoid leukemia (*e.g.*, B-cell precursor B-lymphoblastic leukemia/lymphoma, Burkitt's lymphoma, high-grade B-cell lymphoma, Burkitt's-like T-cell precursor T- 30 lymphoblastic leukemia/lymphoma). In a specific embodiment, the biological composition can ameliorate, reduce, manage, or eliminate the symptoms associated with lymphoma include painless swelling in one or more of the lymph nodes of the neck, collarbone region, armpits, or groin., chest pain, coughing, fatigue, shortness of

breath, fever, drenching night sweats, weight loss, fatigue, appetite loss, red patches on the skin, and severely itchy skin, often affecting the legs/feet.

According to the invention, the biological composition useful for treatment of lymphoma cancer comprises activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.562 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method of preparing said biological composition comprises in any order the steps of:

- (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,977 MHz and a field strength of 282 mV/cm;
 - (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 7,998 MHz and a field strength of 277 mV/cm;
 - (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 10,029 MHz and a field strength of 387 mV/cm;
 - (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,837 MHz and a field strength of 442 mV/cm; and
 - (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,862 MHz and a field strength of 325 mV/cm;
- and after the last of the first five steps, the following steps in any order:
- (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,837 MHz and a field strength of 391 mV/cm; and
 - (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,862 MHz and a field strength of 315 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 60 μ g of vitamin H, 40 μ g of vitamin B₆, 40 μ g of vitamin B₁₂, 0.20 g of KH₂PO₄, 0.20 g of MgSO₄·7H₂O, 0.20 g of NaCl, 0.20 g of CaSO₄·2H₂O, 3.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- 5 (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,837 MHz and a field strength in the range of 230 to 450 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 442 mV/cm, 341 mV/cm, and 244 mV/cm; and
- 10 (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,862 MHz and a field strength in the range of 120 to 380 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 350 mV/cm, 224 mV/cm, and 137 mV/cm.

15 Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice., and soybean juice.

Leukemia

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a
20 subject suffering from leukemia. As used herein, the term "leukemia" includes but is not limited to acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, and hairy cell leukemia. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with leukemia include a weakened immune
25 system, infections, fevers, decrease in red blood cells and platelets, weakness, fatigue, loss of appetite, loss of weight, swollen or tender lymph nodes, liver, or spleen, easy bleeding or bruising, tiny red spots (called petechiae) under the skin, swollen or bleeding gums, sweating (especially at night), bone or joint pain, headaches, vomiting, confusion, loss of muscle control, and seizures.

30 According to the invention, the biological composition useful for treatment of leukemia comprises activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.11 yeast cells cultured in a series of alternating electromagnetic

(EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

- 5 (a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 9,978 MHz and a field strength of 217 mV/cm;
- (b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 11,484 MHz and a field strength of 207 mV/cm;
- 10 (c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,196 MHz and a field strength of 238 mV/cm;
- (d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,565 MHz and a field strength of 244 mV/cm; and
- 15 (e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,612 MHz and a field strength of 262 mV/cm;

and after the last of the first five steps, the following steps in any order:

- 20 (f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,565 MHz and a field strength of 265 mV/cm; and
- (g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,612 MHz and a field strength of 278 mV/cm.

25 Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μ g of vitamin B₁₂, 30 μ g of vitamin B₆, 20 μ g of vitamin D, 20 μ g of vitamin H, 30 ml of bovine calf serum, 0.25 g of MgSO₄·7H₂O, 0.30 g of NaCl, 0.20 g of CaSO₄·2H₂O, 4.0 g of CaCO₃·5H₂O, 2.5 g of peptone, and 1,000 ml of autoclaved water.

30 Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

- 5 (h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,565 MHz and a field strength in the range of 170 to 290 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 270 mV/cm, 277 mV/cm, and 184 mV/cm; and
- 10 (i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,612 MHz and a field strength in the range of 250 to 320 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 296 mV/cm, 318 mV/cm, and 267 mV/cm.
- Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

EXAMPLES: ANIMAL STUDIES

15 Various animal models of human cancer were used to illustrate the benefits of the biological compositions of the invention. Specifically, the effects of the biological compositions on the growth of tumors and the survival of the animals were studied.

The biological compositions used in the following animal studies comprised 10^8 per ml of activated and conditioned yeast cells that were prepared by
20 the methods described above.

1. Lung Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *S. cerevisiae* IFFI1345 cells. The Lewis lung cancer model was used to study the growth of tumors
25 in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Mouse Model

The animals used to generate the Lewis lung cancer cells for the experiments were C57/B1 mice, 6 to 8 weeks old. Lewis lung carcinoma (obtainable from the National Cancer Institute, Bethesda, MD) in a suspension containing about
30 10^6 viable tumor cells was injected subcutaneously in six animals. The animals were allowed to grow for 21 days. The animals that showed robust growth of the tumor

were used as donors. The tumor was removed from a mouse and minced aseptically in 4 ml of Hank's solution. The suspension of tumor cells were injected into a healthy batch of C57/B1 mice that were 6 to 8 weeks old; each mouse receiving 0.2 ml of the tumor cell suspension.

5 The mice injected with tumor cells were divided into 3 experimental groups of ten mice per group and one control group. The three groups were triplicated (*i.e.*, using a total of 90 mice in the experimental groups). In group A, the mice received 0.3 ml of the biological composition once per day. In group B, the mice received 0.5 ml of the biological composition once per day. In group C, the
10 mice received 0.5 ml of physiological saline once per day. A fourth group of mice, group D, which did not receive tumor cells, was given 0.3 ml of physiological saline per day.

 The mice received the biological compositions or saline on the same day as the tumor cells were transplanted. The mice in group D also started receiving
15 saline on the same day as the other three groups. The biological composition or saline were administered orally by a feeding tube for 24 consecutive days. On the 25th day from tumor inoculation, the mice were sacrificed and the weight of the lungs as well as the weight of the tumor were determined by standard techniques.

 Table 7 shows the differences in the weight of the lungs and tumors of
20 the mice in the various treatment and control groups.

Table 7

Group	mean weight of lungs and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
A	209 ± 12	7.2 ± 3
B	158 ± 9	2.6 ± 2
C	1056 ± 124	27.3 ± 7
D	151 ± 14	not applicable

 The mice bearing Lewis cancer cells that received 0.5 ml of the biological composition of the invention (group B) showed the least deviation in the
25 weight of lungs as compared to healthy mice not injected tumor cells (group D). The

mice of group B also had less tumor mass as compared to mice that did not receive treatment (group C) as well as the mice in group A (0.3 ml per day).

Survival Time in Mouse Model

The animals used to generate the Lewis lung cancer cells for the experiments were C57/B1 mice, 6 weeks old. Lewis lung carcinoma (obtainable from the National Cancer Institute, Bethesda, MD) in a suspension containing about 10^8 viable tumor cells was injected subcutaneously in five animals. The animals were allowed to grow for 15 days. The animals that showed robust growth of the tumor were used as donors. The tumor was removed from a mouse and minced aseptically in 4 ml of Hank's solution to form a suspension of tumor cells. 0.1 ml of the cell suspension were injected intramuscularly into each mouse. Healthy C57/B1 mice that were 6 weeks old were used.

The mice injected with tumor cells were divided into 3 experimental groups and one control group of ten mice per group. The groups were in triplicates, *i.e.*, using a total of 120 mice for the four treatments. In group A, the mice received 0.3 ml of the biological composition once per day. In group B, the mice received 0.5 ml of the biological composition once per day. In group C, the mice received 0.5 ml of physiological saline once per day. The activated and conditioned yeast cells were at a concentration of 10^8 per ml of the biological composition. A fourth group of control mice, group D, which did not receive tumor cells, was given 0.3 ml of physiological saline per day.

The mice received the biological compositions or saline 15 days after the tumor cells were transplanted. The mice in group D also started receiving saline on the same day as the other three groups. The biological composition or saline were administered orally by a feeding tube for 60 consecutive days. The mice were observed over a year from the day of tumor inoculation.

An additional set of experiments were also conducted as described above except that the mice were fed the biological composition or saline for 90 days.

Table 8 shows the number of mice in the various treatment and control group that survived the tumor injection over a period of 12 months. Each of the 30 mice in each group received 60 consecutive days of either saline or biological composition of the invention.

Table 8 Number of live animals remaining in the groups after 60 days of treatment

Time after cessation of treatment	Group A	Group B	Group C	Group D
0 month	30	29*	24	30
1 month	30	29	14	30
2 months	30	29	4	30
3 months	30	29	0	30
4 months	30	29	0	30
5 months	29*	29	0	30
6 months	29	29	0	30
7 months	29	29	0	30
8 months	29	29	0	30
9 months	29	28*	0	30
10 months	29	28	0	30
11 months	29	28	0	30
12 months	29	28	0	30

* The mice died of other causes unrelated to the lung cancer.

The above experiment was repeated with mice receiving the different treatments for 90 consecutive days instead of 60 days. Table 9 shows the number of mice in the various treatment and control group that survived the tumor injection over a period of 12 months.

Table 9 Number of live animals remaining in the groups after 90 days of treatment

Time after cessation of treatment	Group A	Group B	Group C	Group D
0 month	30	30	22	29
1 month	30	30	11	29
2 months	30	30	6	29

3 months	30	30	1	29
4 months	30	30	0	29
5 months	30	30	0	29
6 months	30	30	0	29
7 months	29*	30	0	29
8 months	29	30	0	29
9 months	29	30	0	29
10 months	29	30	0	29
11 months	29	30	0	29
12 months	29	30	0	29

* The mice died of other causes unrelated to the lung cancer.

2. Nasopharyngeal Cancer

5 The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.116 cells. The Wistar rat model and a mouse model of human nasopharyngeal cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

10

Tumor Growth in Wistar Rat Model

The animals used to generate the nasopharyngeal cancer cells for the experiments were Wistar rats, male and female, 190 to 210 gram in body weight. The rats were starved for 24 hours before the surgery.

15

Human nasopharyngeal cancer samples (obtainable from Cancer Institute, The Chinese Academy of Medical Sciences, Beijing, China) were carefully selected before, during, and after surgery in order to ensure engraftment occurred at the original position in the rat. The nasopharyngeal cancer samples were obtained from patients who have not received any radiation, chemotherapy or immune enhancement treatment. Nasopharyngeal cancer samples were divided into sections

20

of about 1 mm³ in size and immediately stored in RPMI-1640 suspension after removal from the human patient.

The animals were generally anaesthetized. The barbiturates was first diluted in saline and then injected at 0.3 to 0.4 ml per animal, or 30 mg per kg body weight into the abdomen of the rats. Under sterile conditions, a 1 cm long opening in the body cavity was cut along the right rib of the rats for engraftment of the tumor. Afterwards, the opening was sutured and the rats were put back into the cage. The animals were allowed to grow for three to five weeks. The animals that showed robust growth of the tumor were used as donors. The tumor was removed from a donor rat and minced aseptically in 4 ml of Hank's solution. The suspension of tumor cells were transplanted into a healthy batch of Wistar rats for experimentation.

The rats transplanted with tumor cells were divided into 4 experimental groups of ten rats per group and one control group. The four experimental groups were triplicated (i.e., using a total of 120 rats in the experimental groups). In group AY, the rats received 2.0 ml of the biological composition once per day. In group NY, the rats received 2.0 ml of the untreated yeast cells once per day. In group TSPA, the rats were injected intravenously with 1.5 mg of thiotepa (TSPA) per kg body weight per day. In group CK1, the rats received 2.0 ml of physiological saline once per day. A fifth group of rats, group CK2, which did not receive tumor cells, was given 2.0 ml of physiological saline per day.

The rats received the biological compositions, untreated yeast cells, TSPA or saline on the same day as the tumor cells were transplanted. The rats in group CK2 also started receiving saline on the same day as the other four groups. The biological compositions, untreated yeast cells and saline were administered orally by a feeding tube and the TSPA by intravenous injection for 21 consecutive days. On the 22nd day from tumor inoculation, the rats were sacrificed and the weight of the rats as well as the weight of the tumor were determined by standard techniques.

Table 10 shows the differences in the weight of the rats and tumors of the rats in the various treatment and control groups.

Table 10

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
-------	--	--

AY	214 \pm 2.2	0.19 \pm 0.21
NY	194 \pm 3.2	2.3 \pm 0.81
TSPA	207 \pm 3.8	1.6 \pm 0.74
CK1	197 \pm 3.6	2.7 \pm 0.84
CK2	215 \pm 3.4	not applicable

The rats bearing nasopharyngeal cancer cells that received 2.0 ml of the biological composition of the invention (group AY) showed the least deviation in weight as compared to healthy rats not injected tumor cells (group CK2). The rats in group AY also had less tumor mass as compared to rats that did not receive treatment (group CK1) as well as the rats in group NY (2.0 ml of untreated yeast cells per day) and the rats in group TSPA (1.5 mg of thiotepa per kg body weight per day).

Survival Time in Wistar Rat Model

The animals were prepared in a similar manner as described immediately above. The rats transplanted with tumor cells were divided into 4 experimental groups of ten rats per group and one control group. The four experimental groups were triplicated (*i.e.*, using a total of 120 rats in the experimental groups). In group 2AY, the rats received 2.0 ml of the biological composition once per day. In group 2NY, the rats received 2.0 ml of the untreated yeast cells once per day. In group 2TSPA, the rats were injected intravenously 1.5 mg of thiotepa (TSPA) per kg body weight per day. In group 2CK1, the rats received 2.0 ml of physiological saline once per day. A fifth group of rats, group 2CK2, which did not receive tumor cells, was given 2.0 ml of physiological saline per day.

The rats received the biological compositions, untreated yeast cells, TSPA or saline on the same day as the tumor cells were transplanted. The rats in group 2CK2 also started receiving saline on the same day as the other four groups. The biological compositions, untreated yeast cells and saline were administered orally by a feeding tube and the TSPA by intravenous injection for 21 consecutive days. The rats were observed over 6 months from the day of tumor inoculation and survival was recorded. The weight of the rats and the weight of the tumor were determined by standard techniques.

Table 11 shows the number of rats in the various treatment and control group that survived the tumor injection over a period of 6 months. Each of the 30 rats in each group received 21 consecutive days of either untreated yeast cells, TSPA, saline or biological compositions of the invention. Table 6 shows the weight of the rats that survived and the weight of their tumors in the various treatment and control groups.

Table 11 Number of live animals remaining in the groups after 21 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2TSPA	Group 2CK1	Group 2CK2
0 month	30	28	30	30	30
1 month	30	24	26	22	30
2 months	30	5	24	3	30
3 months	30	0	17	0	30
4 months	30	0	7	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 12

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	226 ± 8.6	132.5 ± 10.3
2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	229 ± 11.8	not applicable

The rats bearing nasopharyngeal cancer cells that received 2.0 ml of the biological composition of the invention (group 2AY) survived for more than 6 months and the tumor never reoccurred. On the contrary, the rats in group 2NY (2.0

ml of untreated yeast cells per day), group 2TSPA (1.5 mg of thiotepa per kg body weight per day) and group 2CK1 (2.0 ml of saline per day) all died after four months from injection of tumor cells.

As in the Example immediately above (i.e. Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer), the rats bearing nasopharyngeal cancer cells that received 2.0 ml of the biological composition of the invention (group 2AY) showed the least deviation in the weight of rats as compared to healthy rats not injected tumor cells (group 2CK2).

10 Tumor Growth in Mouse Model

The animals used to generate the nasopharyngeal cancer cells for the experiments were nasopharyngeal cancer mice (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 6 to 8 weeks old, male and female, 18 to 20 gram in body weight. Highly differentiated nasopharyngeal cancer
15 were obtained from the Institute of Medicine, Cancer Laboratory of China. The animals were injected with a suspension containing about 10^7 viable tumor cells subcutaneously and allowed to grow for 5 days.

The mice injected with tumor cells were divided into 4 experimental groups of ten mice per group and one control group. The four experimental groups
20 were triplicated (i.e., using a total of 120 mice in the experimental groups). In group AY, the mice received 0.3 ml of the biological composition once per day. In group NY, the mice received 0.3 ml of the untreated yeast cells once per day. In group CTX, the mice were injected subcutaneously with 30 mg of cyclophosphamide (CTX) per kg body weight per day. In group CK1, the mice received 0.3 ml of physiological
25 saline once per day. A fifth group of mice, group CK2, which did not receive tumor cells, was given 0.3 ml of physiological saline per day.

The mice received the biological compositions, untreated yeast cells, CTX or saline on the same day as the tumor cells were transplanted. The mice in group CK2 also started receiving saline on the same day as the other four groups. The
30 biological compositions, untreated yeast cells and saline were administered orally by a feeding tube and the CTX by intravenous injection for 30 consecutive days. On the 31st day from tumor inoculation, the mice were sacrificed and the weight of the mice as well as the weight of the tumor were determined by standard techniques. The results are shown below.

Table 13

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	20.3 \pm 1.7	0.12 \pm 0.06
NY	18.5 \pm 2.8	0.95 \pm 0.23
CTX	19.7 \pm 2.4	0.86 \pm 0.13
CK1	18.1 \pm 2.6	0.98 \pm 0.21
CK2	20.6 \pm 2.1	not applicable

Survival Time in Mouse Model

The animals were prepared in a similar manner as described immediately above. The mice injected with tumor cells were divided into 4 experimental groups of ten mice per group and one control group. The four experimental groups were triplicated (i.e., using a total of 120 mice in the experimental groups). In group 2AY, the mice received 0.3 ml of the biological composition once per day. In group 2NY, the mice received 0.3 ml of the untreated yeast cells once per day. In group 2CTX, the mice were injected intravenously with 30 mg of cyclophosphamide (CTX) per kg body weight per day. In group 2CK1, the mice received 0.3 ml of physiological saline once per day. A fifth group of mice, group 2CK2, which did not receive tumor cells were given 0.3 ml of physiological saline per day.

The mice received the biological compositions, untreated yeast cells, CTX or saline on the same day as the tumor cells were transplanted. The mice in group 2CK2 also started receiving saline on the same day as the other four groups. The biological compositions, untreated yeast cells and saline were administered orally by a feeding tube and the CTX by intravenous injection for 30 consecutive days. The mice were observed over 6 months from the day of tumor inoculation and survival was recorded. The weight of the mice and the weight of the tumor were determined by standard techniques.

Table 14 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	29	30	27	30
1 month	30	22	27	19	30
2 months	30	7	17	3	30
3 months	30	0	11	0	30
4 months	30	0	3	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 15

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.3 \pm 1.6	178.5 \pm 9.3
2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	20.8 \pm 1.2	not applicable

3. Esophageal Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.375 cells. The Wistar rat model and a mouse model of human esophageal cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

10

Tumor Growth in Wistar Rat Model

According to numerous animal studies N-methyl-N-benzyl nitrosamine (MBNA) is a well known effective trigger of esophageal cancer in Wistar rats.

Accordingly, this experiment used MBNA in setting up a Wistar rat model of human esophageal cancer. Detailed description of the use of MBNA to induce esophageal cancer can be found in *e.g.*, Lu S.X., 1989, *Zhonghua Zhong Liu Za Zhi* 11(6):401-3; Craddock V.M. and Driver H.E., 1987, *Carcinogenesis* 8(8):1129-32, each of which
 5 is incorporated herein by reference in its entirety.

The animals used in the experiments were Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), male and female, with an average body weight of about 150 to 180 gram. The rats were given water containing 100 ppm MBNA and fed a diet containing MBNA such that each rat
 10 received 0.75 mg MBNA (obtainable from Beijing Chemical Reagent Company, China) per kg body weight per day for 30 days. All the animals essentially developed esophageal cancer.

The rats that were fed with MBNA for 30 days were divided into 4 experimental groups of ten rats per group and one control group. The four
 15 experimental groups were triplicated (*i.e.*, using a total of 120 rats in the experimental groups). In group AY, the rats received 1.5 ml of the biological composition in two doses per day. In group NY, the rats received 1.5 ml of the untreated yeast cells in two doses per day. In group VDS, the rats were injected intravenously with 3.0 mg of vindesine (VDS) per kg body weight once a week for four weeks. In group CK1, the
 20 rats received 1.5 ml of physiological saline in two doses per day. A fifth group of rats, group CK2, which did not receive tumor cells, was given 1.5 ml of physiological saline in two doses per day.

The biological compositions, untreated yeast cells and saline were administered orally by a feeding tube and the VDS by intravenous injection for 30
 25 consecutive days. On the 31st day from tumor inoculation, the rats were sacrificed.

Table 16

Group	mean weight of rats and standard deviation (g)	mean weight of esophagus and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
AY	186 ± 14	122 ± 19	109 ± 11
NY	168 ± 17	174 ± 32	877 ± 18

VDS	173 ± 15	179 ± 27	281 ± 31
CK1	166 ± 19	176 ± 27	889 ± 26
CK2	188 ± 15	109 ± 12	not applicable

Survival Time in Wistar Rats

The experiment was carried out in a manner similar to that described immediately above, except that the survival time over a period of over 6 months was
5 observed.

Table 17 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VDS	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	27	30	30	30
2 months	30	22	30	24	30
3 months	30	17	30	19	30
4 months	30	7	21	7	30
5 months	30	0	3	0	30
6 months	30	0	0	0	30

Table 18

Group	mean weight of rats and standard deviation (g)	mean weight of esophagus and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	203 ± 22	112 ± 17	64.4 ± 0.7
2NY	all animals dead	all animals dead	all animals dead
2VDS	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	211 ± 17	101 ± 16	not applicable

Tumor Growth in TA1 Mouse Model

Numerous animal studies have reported the use of murine models in the study of human esophageal cancer. There is almost a 100% success rate for transplanting esophageal tumor SGA-73 cells in mice. Detailed description of the esophageal tumor cell line SGA-73 can be found in Ding R. *et al.*, Esophageal Cancer Experimental Research, Ren Ming Wei Shen Publisher, 1980, p. 36, which is incorporated herein by reference in its entirety.

The animals used for the experiments were TA1 mice (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 6 to 8 weeks old, male and female, with an average body weight of about 18 to 22 gram. The esophageal cancer cell line SGA-73 (obtainable from the Beijing Institute of Chinese Medicine and Pharmacology, Beijing, China) in a suspension containing about 10^7 viable tumor cells (about 0.2 ml culture suspension) was injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the Tumor Growth in Mouse Model section for the nasopharyngeal cancer.

Table 19

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	18.8 ± 2.7	131 ± 17
NY	17.7 ± 3.2	472 ± 23
CTX	18.2 ± 2.8	384 ± 31
CK1	17.6 ± 2.8	534 ± 25
CK2	19.4 ± 2.4	not applicable

Survival Time in Mouse Model

This experiment was carried out in a similar manner as described above in the Tumore Growth in Mouse Model for esophageal, except that the survival time of the mice was recorded for a period over 6 months. In addition, the dosages used in groups 2AY, 2NY, 2CK1 and 2CK2 were increased to 0.5 ml once per day

and the dosage for 2CTX was increased to 45 mg of cyclophosphamide (CTX) per kg body weight per day.

Table 20 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	24	30	27	30
2 months	30	16	28	21	30
3 months	30	7	22	14	30
4 months	30	2	18	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 21

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.4 ± 27.2	91.9 ± 0.7
2NY	all animals dead	all animals dead
2CTX	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.8 ± 22.2	not applicable

5

4. Stomach Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.14 cells. The Wistar rat model and a mouse model of human stomach cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Wistar Rat Model

Numerous animal studies have reported the use of Wistar rats in the study of treating stomach cancer. 1,2-dimethylhydrazine (DMH) is a well-known effective trigger of stomach cancer in rats. Accordingly, this experiment used DMH in designing Wistar rat model of human stomach cancer. Detailed description of the use of 1,2-dimethylhydrazine to induce stomach cancer can be found in Watanabe H. *et al.*, 1999, *Jpn J Cancer Res.* 90(11):1207-11, which is incorporated herein by reference in its entirety.

The animals used in the experiments were Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). The stomach cancer cell line (obtainable from the First Military Medical University of China, Guangzhou, China) was generated in 1991 from a Wistar rat stomach cancer which was induced by subcutaneous injection of DMH. The rats were injected subcutaneously with 2.5×10^6 stomach cancer cells per animal.

The experiment was carried out in a manner similar to that described above in the Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except that a dosage of 0.8 ml per day was used for groups AY, NY, CK1 and CK2, and a group ADM was introduced in lieu of group TSPA, wherein the rats were injected intravenously with 10^5 units of doxorubicin (adriamycin, ADM) per kg body weight per day.

Table 22

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	196.6 ± 11.7	232.7 ± 9.6
NY	177.7 ± 14.5	689.6 ± 13.1
ADM	181.8 ± 12.8	446.4 ± 13.6
CK1	178.5 ± 12.5	698.5 ± 13.1
CK2	204.6 ± 13.7	not applicable

Survival Time in Wistar Rat Model

This experiment was carried out in a similar manner as described immediately above, except that survival time for a period over 6 months was observed. In addition, the dosages for groups 2AY, 2NY, 2CK1, and 2CK2 were increased to 1.2 ml per day while the dosage for 2ADM was increased to 1.5×10^5 units of doxorubicin (adriamycin, ADM) per kg body weight per day.

Table 23 Number of live animals remaining in the groups after 21 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2ADM	Group 2CK1	Group 2CK2
0 month	30	29	30	30	30
1 month	30	25	30	28	30
2 months	30	21	30	18	30
3 months	30	18	22	11	30
4 months	30	11	17	7	30
5 months	30	0	13	0	30
6 months	30	0	0	0	30

Table 24

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	213.4 \pm 13.9	89.9 \pm 0.7
2NY	all animals dead	all animals dead
2ADM	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	222.5 \pm 11.3	not applicable

Tumor Growth in Mouse Model

Numerous animal studies have reported the use of murine models in the study of treating stomach cancer. Methylcholanthrene (MC) is a well-known effective trigger of stomach cancer in mice. Accordingly, this experiment used MC in

designing TA2 mouse model of human stomach cancer. Detailed description of the use of methylcholanthrene to induce stomach cancer can be found in Wang X.H. *et al.*, 1984, *Chin Med J (Engl)* 97(3):215-22, which is incorporated herein by reference in its entirety.

5 The animals used for the experiments were TA2 mice, 6 to 8 weeks old (obtainable from the Second Military Medical University, Shanghai, China). Both male and females with an average weight of about 18 to 20 gram were used. The stomach cancer cell line S784 (obtainable from MC-treated TA2 mouse provided by the Tianjian Medical University, China) in a suspension containing about 1.2×10^7
10 viable tumor cells (about 0.2 ml cell culture) was injected subcutaneously into the animals. The mice injected with tumor cells were kept for 5 days.

The experiment was carried in a manner similar to that described in section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group ADM was used in place of group CTX, wherein the mice were injected
15 intravenously with 10^5 units of doxorubicin (adriamycin, ADM) per kg body weight per day.

Table 25

Group	mean weight of mice and standard deviation (g)	mean weight of stomach and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
AY	21.2 ± 0.6	412 ± 18	132 ± 9
NY	17.7 ± 0.5	778 ± 19	489 ± 31
ADM	19.8 ± 0.8	621 ± 17	346 ± 36
CK1	17.5 ± 0.5	776 ± 15	498 ± 31
CK2	21.6 ± 0.7	368 ± 17	not applicable

Survival Time in Mouse Model

20 This experiment is otherwise similar to that described immediately above, except that the animals were observed for a period of more than 6 months and their survival time was recorded. In addition, the dosages for groups 2AY, 2NY, 2CK1, and 2CK2 were increased to 0.5 ml per day while the dosage for 2ADM was

increased to 1.5×10^5 units of doxorubicin (adriamycin, ADM) per kg body weight per day.

Table 26 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2ADM	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	26	30	24	30
2 months	30	17	27	13	30
3 months	30	3	16	0	30
4 months	30	0	7	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 27

Group	mean weight of mice and standard deviation (g)	mean weight of stomach and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	21.4 ± 0.9	371 ± 12	72.2 ± 0.6
2NY	all animals dead	all animals dead	all animals dead
2ADM	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	22.5 ± 1.3	369 ± 14	not applicable

5

5. Colorectal Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.1396 cells. The Wistar rat model and a mouse model of human colorectal cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Wistar Rat Model

According to numerous animal studies, dimethyl hydrazine (DMH) is a well known effective trigger of colorectal cancer in Wistar rats. Accordingly, this experiment used DMH in setting up a Wistar rat model of human colorectal cancer. Detailed description of the use of dimethyl hydrazine to induce colorectal cancer can be found in Madarnas P. *et al.*, 1992, *Anticancer Res.* 12(1):113-7, which is incorporated herein by reference in its entirety.

A DMH mixture was prepared by mixing 400 mg DMH with 100 ml saline and 37 mg ethylene diamine tetraacetate (EDTA). The pH of the mixture was then adjusted to 6.5 using 0.1 N NaOH.

The animals used in the experiments were Wistar rats (obtained from the Chinese Academy of Military Medical Sciences, Beijing, China), male and female, with an average body weight of about 180 to 200 gram. The rats were injected subcutaneously with 21 mg of DMH mixture (as prepared above) per kg body weight once a week for 21 weeks.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except that a group AM was used in lieu of Group TSPA, wherein the rats were injected subcutaneously with 10^5 units of amycin (AM) per kg body weight per day. The animals were treated for 30 days and was sacrificed on the 31st day from tumor inoculation.

Table 28

Group	mean weight of rats and standard deviation (g)	mean weight of colorectum and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
AY	207 ± 11	1,005 ± 21	107 ± 23
NY	189 ± 15	1,564 ± 39	655 ± 47
AM	202 ± 12	1,214 ± 32	304 ± 33
CK1	187 ± 13	1,546 ± 37	626 ± 42
CK2	197 ± 18	893 ± 22	not applicable

Survival Time in Wistar Rats

The experiment was carried out in a similar manner as described immediately above, except that the rats were observed for a period of over 6 months from the day of tumor inoculation and the survival time was recorded.

5 Table 29 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2AM	Group 2CK1	Group 2CK2
0 month	30	30	30	28	30
1 month	30	26	30	23	30
2 months	30	11	26	17	30
3 months	30	0	22	9	30
4 months	30	0	12	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 30

Group	mean weight of rats and standard deviation (g)	mean weight of colorectum and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	226 ± 14	976 ± 17	4.2 ± 1.2
2NY	all animals dead	all animals dead	all animals dead
2AM	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	228 ± 17	917 ± 21	not applicable

Tumor Growth in Mouse Model

Numerous animal studies have reported the use of BALB/c mouse models in the study of human colorectal cancer. There is almost a 100% success rate for transplanting colorectal tumor Ca-26 cells in mice.

The animals used for the experiments were BALB/c mice (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 6 to 8 weeks old. Both males and females with average body weight of about 15 to 18 gram were used. About 10^6 viable tumor cells of the colorectal cancer cell line Ca-26
 5 (about 0.2 ml culture suspension) were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group AM was used in lieu of Group CTX, wherein the mice were injected subcutaneously with 10^5 units of amycin (AM) per kg body weight per day.

10

Table 31

Group	mean weight of mice and standard deviation (g)	mean weight of colorectum and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	17.7 ± 2.0	379 ± 17	69 ± 13
2NY	17.3 ± 1.3	641 ± 29	277 ± 27
2AM	17.4 ± 1.5	587 ± 23	247 ± 29
2CK1	16.5 ± 1.7	623 ± 31	286 ± 33
2CK2	17.8 ± 2.1	326 ± 14	not applicable

Survival Time in Mouse Model

This experiment was carried out in a similar manner as described immediately above, except that the mice were observed for a period of over 6 months
 15 from the day of tumor inoculation and the survival time was recorded.

Table 32 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2AM	Group 2CK1	Group 2CK2
0 month	30	29	30	30	30
1 month	30	23	30	27	30
2 months	30	0	27	0	30

3 months	30	0	21	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 33

Group	mean weight of rats and standard deviation (g)	mean weight of colorectum and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	18.4 ± 1.4	354 ± 16	23.6 ± 0.9
2NY	all animals dead	all animals dead	all animals dead
2AM	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	18.7 ± 1.2	347 ± 19	not applicable

6. Pancreatic Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain IFFI 1413 cells. Two mouse models of human pancreatic cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

10 Tumor Growth in Mouse Model

Numerous animal studies have reported the use of BALB/c mice in the study of treating pancreatic cancer. The use of orthotopic transplant technique has been highly successful in the development of murine models of human pancreatic cancer.

15 The animals used to generate the pancreatic cancer cells for the experiments were BALB/c mice, both male and female with an average body weight of about 18 to 20 gram (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). The pancreatic tumor cells were isolated from clinical

biopsy samples in 1987 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China).

The animals were starved for 24 hours before the experiment. A suspension containing about 1×10^6 pancreatic cancer tumor cells (about 0.2 ml culture suspension) was transplanted into the donors animals at the thorax by injection. Animals that showed robust growth of the tumor were used.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group VDS was used in lieu of Group CTX, wherein the mice were injected intravenously with 3 mg of vindesine (VDS) per kg body weight once a week for four weeks.

Table 34

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	19.8 ± 2.5	0.44 ± 0.3
NY	17.7 ± 3.2	2.86 ± 0.9
VDS	18.9 ± 3.4	1.37 ± 0.7
CK1	17.6 ± 3.6	2.84 ± 0.9
CK2	20.3 ± 2.4	not applicable

Survival Time in Mouse Model

The experiment was conducted in a similar manner as described immediately above, except that the mice were observed for a period of over 6 months from the day of tumor inoculation and the survival time was recorded.

Table 35 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VDS	Group 2CK1	Group 2CK2
0 month	30	30	30	28	30
1 month	30	27	30	22	30

2 months	30	17	27	6	30
3 months	30	0	27	0	30
4 months	29	0	11	0	30
5 months	29	0	0	0	30
6 months	29	0	0	0	30

Table 36

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.7 ± 3.5	78.3 ± 14.2
2NY	all animals dead	all animals dead
2VDS	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.3 ± 2.6	not applicable

Tumor Growth in Kun Ming Mouse Model

Numerous animal studies have reported the use of murine models in the study of treating pancreatic cancer. There is a 100% success rate for transplanting mice with the pancreatic tumor type MPC-83. Detailed description of the transplantable mouse pancreatic cancer cell line MPC-83 can be found in Hu M.Y., 1968, Zhonghua Zhong Liu Za Zhi (Chinese) 8(1):1-3, which is incorporated herein by reference in its entirety.

The animals used for the experiments were kun ming mice, 6 to 8 weeks old (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). Both male and females with an average weight of about 15 to 18 gram were used. The transplantable mouse pancreatic cancer cell line MPC-83 (obtainable from Kun-Ming Medical University, Kun-Ming, China) in a suspension containing about 1.2×10^7 viable tumor cells (about 0.2 ml culture suspension) was injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except

that a group MMC was used in lieu of Group CTX, wherein the mice were injected intravenously with 10^5 units of mitomycin C (MMC) per kg body weight per day.

Table 37

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	19.2 ± 2.2	0.9 ± 0.4
NY	17.4 ± 2.4	3.3 ± 1.5
MMC	18.3 ± 1.5	2.8 ± 1.2
CK1	17.6 ± 2.4	3.6 ± 1.7
CK2	20.3 ± 2.3	not applicable

5 Survival Time in Kun Ming Mouse Model

The experiment was carried out similar to the study described immediately above, with the exception that the mice were observed for a period of over 6 months from the day of tumor inoculation and the survival time was recorded. Moreover, the dosage was increased to 0.5 ml per day for 2AY, 2NY, 2CK1 and 10 2CK2 groups. For the MMC group, the dosage was increased to 1.5×10^5 units of mitomycin C (MMC) per kg body weight per day.

Table 38 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2MMC	Group 2CK1	Group 2CK2
0 month	30	27	30	24	30
1 month	30	0	27	0	30
2 months	30	0	21	0	30
3 months	30	0	7	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30

6 months	30	0	0	0	30
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Table 39

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.5 ± 1.6	104.1 ± 16.7
2NY	all animals dead	all animals dead
2MMC	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.3 ± 2.6	not applicable

7. Liver Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.503 cells. Two mouse models of human liver cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

10 Tumor Growth in Swiss Mouse Model

Numerous animal studies have reported the use of Swiss mice in the study of treating liver cancer. The use of orthotopic transplant technique has been highly successful in the development of mouse models of human liver cancer.

The animals used to generate the liver cancer cells for the experiments were Swiss mice about 5 weeks old (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). Both male and female Swiss mice with an average weight of about 18 to 20 gram were used in the following experiment. The mice were starved for 24 hours before the surgery. Liver tumor tissues were obtained from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China. Animal preparation was carried out in a manner similar to that described above in the nasopharyngeal cancer section.

The experimental was performed in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer.

Table 40

Group	mean weight of mice and standard deviation (g)	mean weight of liver and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
AY	21 \pm 0.7	1,098 \pm 21	107 \pm 7
NY	18.2 \pm 0.8	1,557 \pm 19	579 \pm 13
CTX	17 \pm 0.9	1,372 \pm 8	249 \pm 9
CK1	19.7 \pm 1.1	1,489 \pm 17	601 \pm 14
CK2	21 \pm 0.5	997 \pm 22	not applicable

5

Survival Time in Swiss Mouse Model

The experiment was carried out similar to the study described immediately above, except that the mice were observed for a period of over 6 months from the day of tumor inoculation and their survival time was recorded.

Table 41 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	26	30	24	30
1 month	30	22	30	16	30
2 months	30	11	27	6	30
3 months	30	0	22	0	30
4 months	30	0	19	0	30
5 months	30	0	7	0	30
6 months	30	0	0	0	30

10

Table 42

Group	mean weight of mice and standard deviation (g)	mean weight of liver and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	22 ± 0.6	988 ± 8	1.1 ± 0.2
2NY	all animals dead	all animals dead	all animals dead
2VDS	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	22 ± 0.8	not applicable	not applicable

Tumor Growth in C₃H Mouse Model

Numerous animal studies have reported the use of murine models in the study of treating liver cancer. Murine transplantable liver tumor type H-22 closely resembles the human liver cancer pathology. Detailed description of the murine ascites hepatoma cell line H-22 can be found in Ling M.Y., 1991, Zhonghua Zhong Liu Za Zhi 13(1):13-5, which is incorporated herein by reference in its entirety. The expression of the alphafetoprotein (AFP) gene is associated with liver cancer, and there is an association between changes in the liver tumor and the level of AFP protein in blood plasma. More information on the use of AFP in the diagnosis of liver cancer can be found in Shi X. et al., 1998, Zhonghua Zhong Liu Za Zhi 20(6):437-439, which is incorporated herein by reference in its entirety.

The animals used to generate the liver cancer cells for the experiments were C₃H mice, 6 to 8 weeks old (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). Both male and females were used. The mice were starved for 24 hours before the surgery. Liver tumor H-22 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) were injected into the mice. Ten days after transplantation, the tumors are visible and the level of AFT in plasma is detectable.

The animals were allowed to grow for 21 days. The animals that showed robust growth of the tumor were used as donors. The tumor was removed from a mouse and minced aseptically in 4 ml of Hank's solution. The suspension of tumor cells were injected into a healthy batch of C₃H mice that were 6 to 8 weeks old; each mouse receiving 0.2 ml of the tumor cell suspension.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer.

Table 43

Group	mean weight of mice and standard deviation (g)	mean weight of liver and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
AY	21.2 ± 0.6	1,142 ± 7	152 ± 6
NY	18.7 ± 0.6	1,687 ± 113	783 ± 102
CTX	18.8 ± 0.7	1,351 ± 9	253 ± 8
CK1	16.2 ± 0.8	1,774 ± 121	767 ± 121
CK2	21.6 ± 0.5	986 ± 7	not applicable

5

Survival Time in C₃H Mouse Model

The experiment was carried out similar to the study described immediately above, except that the mice were observed for a period of over 6 months from the day of tumor inoculation and their survival time was recorded.

Table 44 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	30	30	28	30
1 month	30	28	27	24	30
2 months	30	17	24	16	30
3 months	30	0	19	0	30
4 months	29	0	11	0	30
5 months	29	0	3	0	30
6 months	29	0	0	0	30

10

Table 45

Group	mean weight of mice and standard deviation (g)	mean weight of liver and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	23.6 \pm 2.2	988 \pm 16	6.1 \pm 0.22
2NY	all animals dead	all animals dead	all animals dead
2VDS	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	23.7 \pm 2.6	983 \pm 14	not applicable

8. Testicular Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.182. The Kun Ming mouse model and the Wistar rat model of human testicular cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Kun Ming Mouse Model

Numerous animal studies have reported the use of kun ming mouse models in the study of human testicular cancer. There is almost a 100% success rate for transplanting testicular tumor HTB-104 cells in mice. The testicular tumor cell line HTB-104 can be ordered from the American Type Culture Collection.

The animals used for the experiments were kun ming mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). Only males 20 to 22 gram in body weight, 6 to 7 months old were used. About 1.2×10^7 viable tumor cells of the testicular cancer cell line HTB-104 (obtained from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension was injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group BLM was used in lie of the CTX group, wherein the mice were injected subcutaneously with 0.5 mg of bleomycin (BLM) per kg body weight per day.

Table 46

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	19.2 ± 2.2	0.77 ± 0.47
NY	17.7 ± 2.5	1.76 ± 0.67
BLM	18.3 ± 2.4	1.13 ± 0.65
CK1	17.6 ± 2.5	1.87 ± 0.78
CK2	19.8 ± 2.6	not applicable

Survival Time in Kun Ming Mouse Model

- The experiment was carried out in a manner similar to the study
- 5 described immediately above, except that the mice were observed for a period of over 6 months from the day of tumor inoculation and the survival time was recorded. In addition, the dosage was increased to 0.5 ml per day for groups 2AY, 2NY, 2CK1 and 2CK2 groups. For group 2BLM, the dosage was increased to 0.8 mg of bleomycin (BLM) per kg body weight per day.

- 10 Table 47 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2BLM	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	22	27	24	30
2 months	30	11	16	13	30
3 months	30	0	0	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 48

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	21.1 \pm 2.4	89.6 \pm 16.5
NY	all animals dead	all animals dead
BLM	all animals dead	all animals dead
CK1	all animals dead	all animals dead
CK2	22.6 \pm 2.6	not applicable

Tumor Growth in Wistar Rat Model

Numerous animal studies have reported the use of Wistar rat models in the study of human testicular cancer. There is very high success rate for transplanting testicular tumor CRL-1973 cells in rats. The testicular tumor cell line CRL-1973 can be ordered from the American Type Culture Collection.

The animals used for the experiments were male Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). About 2×10^7 viable tumor cells of the testicular cancer cell line CRL-1973 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension was injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except the dosages for groups AY, NY, CK1 and CK2 were decreased to 0.8 ml, and a group VLB was introduced in lieu of group TSPA, wherein the rats were injected intravenously with 0.4 mg of vinblastine (VLB) per kg body weight per day. Thirty (30) days of consecutive treatment was performed and the rats were sacrificed on the 31st day from tumor inoculation.

Table 49

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	193.7 \pm 7.8	0.87 \pm 0.34

NY	186.7 ± 8.4	3.55 ± 2.45
VLB	187.5 ± 8.5	2.67 ± 2.65
CK1	183.7 ± 8.6	3.56 ± 2.46
CK2	202.4 ± 8.6	not applicable

Survival Time in Wistar Rat Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 1.2 ml and, for group VLB, the dosage was increased to 0.55 mg of vinblastin (VLB) per kg body weight per day. The survival time for a period of over six (6) months was recorded.

Table 50 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VLB	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	28	30	30	30
2 months	30	0	22	25	30
3 months	30	0	9	4	30
4 months	30	0	1	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 51

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	206.5 ± 10.6	287.5 ± 32.3
NY	All animals dead	all animals dead
VLB	All animals dead	all animals dead

CK1	All animals dead	all animals dead
CK2	212.7 ± 12.5	not applicable

9. Prostate Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces*
 5 *carlsbergensis* Hansen strain AS2.440. The nude mouse model of human prostate cancer was used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Nude Mouse Model

10 Numerous animal studies have reported the use of nude mouse models in the study of treating prostate cancer. See, for example, People's Republic of China Pharmacopia, 2000, Chinese Health Science Publisher. The use of orthotopic transplant technique has been highly successful in the development of mouse models of human prostate cancer.

15 The animals used to generate the prostate cancer cells for the experiments were male nude mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China) with an average weight of about 15 to 18 gram. Fresh prostate tumor cells (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) were divided into sections of size 1-2
 20 mm³ and immediately stored in RPMI-1641 suspension after removal from the human patient.

The animal preparation was carried out in a manner similar to that described in the section of the nasopharyngeal cancer. The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse model
 25 for the nasopharyngeal cancer, except that a group TSPA was introduced in lieu of group CTX, wherein the mice were injected intravenously with 1.5 mg of thiotepa (TSPA) per kg body weight per day.

Table 52

Group	mean weight of mice and	mean weight of tumor nodules and standard
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	standard deviation (g)	deviation (mg)
AY	17.1 ± 1.4	0.6 ± 0.7
NY	16.5 ± 1.6	3.3 ± 2.3
TSPA	16.2 ± 1.7	2.4 ± 1.2
CK1	16.3 ± 1.3	3.6 ± 2.4
CK2	17.5 ± 1.5	not applicable

Survival Time in Nude Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2TSPA, the dosage was increased to 2.2 mg of thiotepa (TSPA) per kg body weight per day. The survival time for a period of over six (6) months was recorded.

Table 53 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2TSPA	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	26	30	21	30
2 months	30	1	30	0	30
3 months	30	0	27	0	30
4 months	30	0	17	0	30
5 months	30	0	5	0	30
6 months	30	0	0	0	30

Table 54

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	18.4 ± 2.4	152.2 ± 21.4

2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	18.6 ± 2.1	not applicable

Tumor Growth in Nude Mouse Model

This experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer.

5

Table 55

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	17.4 ± 1.4	0.7 ± 0.5
NY	16.7 ± 1.2	4.3 ± 2.2
CTX	16.7 ± 1.7	3.3 ± 1.7
CK1	16.5 ± 1.3	4.1 ± 1.8
CK2	17.6 ± 1.5	not applicable

Survival Time in Nude Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2CTX, the dosage was increased to 45 mg of cyclophosphamide (CTX) per kg body weight per day. Six (6) months of survival time was recorded.

Table 56 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	24	30	16	30

2 months	30	0	30	1	30
3 months	30	0	27	0	30
4 months	30	0	22	0	30
5 months	30	0	17	0	30
6 months	30	0	0	0	30

Table 57

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	18.8 ± 1.2	157.5 ± 11.3
2NY	All animals dead	all animals dead
2TSPA	All animals dead	all animals dead
2CK1	All animals dead	all animals dead
2CK2	19.2 ± 1.6	not applicable

10. Ovarian Cancer

5

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.502 cells. Two mouse models of human ovarian cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

10

Tumor Growth in Female Mouse Model

Numerous animal studies have reported the use of orthotopic transplant technique in female mouse models to study the treating of ovarian cancer. See, for example, Animals for Experiments and Experimental Techniques, Chinese Medical Publisher, 1997. The use of orthotopic transplant technique has been highly successful in the development of mouse models of human ovarian cancer.

15

The animal preparation was carried out in a manner similar to that described in the section of the nasopharyngeal cancer. The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group TSPA was used in lieu of Group CTX, wherein the mice were injected intravenously with 1.5 mg of thiotepa (TSPA) per kg body weight per day.

Table 58

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	23 ± 1.6	0.7 ± 0.5
NY	20 ± 2.5	3.9 ± 1.3
TSPA	21 ± 2.1	3.1 ± 0.8
CK1	21 ± 2.2	3.7 ± 1.4
CK2	23 ± 2.7	not applicable

Survival Time in Female Mouse Model

This experiment was otherwise similar to that described immediately above, except that the animals' survival time for a period of over 6 months was recorded.

Table 59 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2TSPA	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	12	24	13	30
2 months	30	2	7	2	30
3 months	30	0	2	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30

6 months	30	0	0	0	30
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Table 60

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.0 ± 4.1	36.3 ± 17
2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	20.0 ± 3.2	not applicable

Tumor Growth in C₅₇BL Mouse Model

The animals used to generate the ovarian cancer cells for the experiments were female C₅₇BL mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), six weeks old, having an average body weight of about 18 to 20 gram. Detailed description of the use of C₅₇BL mice can be found in Roby K.F. et al., 2000, Carcinogenesis 21(4):585-91, which is incorporated herein by reference in its entirety. Ovarian tumor cells (cell line obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) were injected subcutaneously into the C₅₇BL mice. The number of tumor cells injected was about 10⁶ units per mouse in a 0.2 ml culture suspension.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer.

Table 61

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	21 ± 2.7	0.7 ± 0.6
NY	18 ± 2.3	4.7 ± 1.6
CTX	20 ± 2.3	3.3 ± 1.4

CK1	19 ± 2.4	4.2 ± 1.9
CK2	22 ± 2.5	not applicable

Survival Time in C₅₇BL Mouse Model

This experiment was otherwise similar to that described immediately above, except that the animals' survival time for a period of over 6 months was
5 recorded.

Table 62 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
1 month	30	29	30	27	30
2 months	30	11	30	7	30
3 months	30	0	23	0	30
4 months	30	0	14	0	30
5 months	30	0	9	0	30
6 months	30	0	0	0	30

Table 63

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	23 ± 2.4	133.7 ± 14.7
2NY	all animals dead	all animals dead
2CTX	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	23 ± 2.9	not applicable

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.441 cells. Two mouse models of human breast cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Mouse Model

The murine transplantable breast cancer cell line Ca615-B was used in the model which resembles human breast cancer pathology. See e.g., In Oncology Basic and Research Protocols (Ed. J. Kao), People's Health Publishing House, Beijing, China, 1998, p.72.

The animals used to generate the breast cancer cells for the experiments were female 615 mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), having an average body weight of about 18 to 20 gram. About 10^6 viable tumor cells of the murine breast cancer cell line Ca615-B (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group TSPA was introduced in lieu of CTX group, wherein the mice were injected intravenously with 1.5 mg of thiotepa (TSPA) per kg body weight per day.

Table 64

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	21 ± 2.3	0.6 ± 0.2
NY	19 ± 2.6	3.3 ± 1.5
TSPA	20 ± 2.4	3.1 ± 1.6
CK1	19 ± 2.5	3.4 ± 1.4
CK2	22 ± 2.1	not applicable

Survival Time in Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2TSPA, the dosage was increased to 2.5 mg of
 5 thiotepa (TSPA) per kg body weight per day. The survival time for a period of over six (6) months was recorded.

Table 65 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2TSPA	Group 2CK1	Group 2CK2
0 month	30	28	30	29	30
1 month	30	0	30	0	30
2 months	30	0	23	0	30
3 months	30	0	11	0	30
4 months	30	0	4	0	30
5 months	29	0	0	0	30
6 months	29	0	0	0	30

Table 66

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	22 ± 1.4	204.2 ± 19.7
2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	22 ± 2.2	not applicable

10

Tumor Growth in Mouse Model

Numerous studies have reported the use of mouse models in finding a treatment for breast cancer. The murine transplantable breast cancer cell line MA

782/5S-B was used in the model which resembles human breast cancer pathology. See e.g., In Oncology Basic and Research Protocols (Ed. J. Kao), People's Health Publishing House, Beijing, China, 1998, p.73.

The animals used to generate the breast cancer cells for the experiments were female mice derived from an outbreed line of mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), having an average body weight of about 18 to 20 gram. About 10^6 viable tumor cells of the breast cancer cell line MA782/5S-B (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in section Tumor Growth in Mouse Model for the nasopharyngeal cancer.

Table 67

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	20.3 ± 1.9	1.7 ± 0.6
NY	19.2 ± 2.4	3.3 ± 2.4
CTX	19.4 ± 2.2	3.1 ± 1.7
CK1	19.1 ± 2.3	3.6 ± 2.2
CK2	21.2 ± 2.1	not applicable

Survival Time in Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2CTX, the dosage was increased to 45 mg of cyclophosphamide (CTX) per kg body weight per day. Six (6) months of survival time was recorded.

Table 68 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
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treatment					
0 month	30	29	30	30	30
1 month	30	16	30	11	30
2 months	30	7	17	3	30
3 months	30	0	0	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 69

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	22 ± 2.2	207.5 ± 11.3
2NY	All animals dead	all animals dead
2TSPA	All animals dead	all animals dead
2CK1	All animals dead	all animals dead
2CK2	22 ± 2.6	not applicable

12. Cervical Cancer

5 The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.444 cells. Two mouse models of human cervical cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

10

Tumor Growth in Mouse Model

The cervical cancer cell line U14 was used in the model which closely resembles the human cervical cancer pathology. Detailed description of the use of

cervical cancer cell line U14 can be found in Tao G. et al., 2001, Chin Med J.

114(6):623-7, which is incorporated herein by reference in its entirety.

The animals used to generate the cervical cancer cells for the experiments were female mice from the 615 strain (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China) having an average body weight of 20 to 22 gram and 4 to 6 months old. About 2×10^6 viable tumor cells of the cervical cancer cell line U14 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that group MMC was used in lie of group CTX, wherein the mice were injected subcutaneously with 0.8 mg of mitomycin C (MMC) per kg body weight per day.

Table 70

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	20.2 ± 2.7	1.3 ± 0.32
NY	18.4 ± 3.3	4.2 ± 2.55
MMC	19.3 ± 3.3	4.4 ± 2.23
CK1	18.9 ± 3.1	4.1 ± 2.64
CK2	20.7 ± 2.2	not applicable

Survival Time in Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2MMC, the dosage was increased to 1.2 mg of mitomycin C (MMC) per kg body weight per day. Six (6) months of survival time was recorded.

Table 71 Number of live animals remaining in the groups after 30 days of treatment

Time after	Group	Group	Group	Group	Group
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cessation of treatment	2AY	2NY	2MMC	2CK1	2CK2
0 month	30	24	30	22	30
1 month	30	4	30	2	30
2 months	30	0	24	0	30
3 months	30	0	13	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 72

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.7 ± 2.4	151.7 ± 17.4
2NY	all animals dead	all animals dead
2MMC	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.6 ± 2.3	not applicable

Tumor Growth in Kun Ming Mouse Model

The cervical cancer cell line U27 was used in the model which closely resembles the human cervical cancer pathology. Detailed description of the use of cervical cancer cell line U27 can be found in Qian S.S. et al., 1987, Zhongguo Yi Xue Ke Xue Yuan Xue Bao 9(1):33-7, which is incorporated herein by reference in its entirety.

The animals used to generate the cervical cancer cells for the experiments were female kun ming mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China) having an average body weight of 20 to 22 gram and 5 to 6 months old. About 1.2×10^7 viable tumor cells of the cervical cancer cell line U27 (obtainable from the Cancer Institute, Chinese Academy of Medical

Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

- The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that group VCR was used in lie of group CTX, wherein the mice were injected intravenously with 3 mg of vinblastine (VCR) per kg body weight per day.

Table 73

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	19.4 ± 2.4	1.53 ± 0.87
NY	17.9 ± 2.3	4.54 ± 2.76
VCR	18.5 ± 3.7	4.77 ± 2.79
CK1	18.3 ± 3.3	4.56 ± 2.47
CK2	20.2 ± 2.6	not applicable

Survival Time in Kun King Mouse Model

- This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2VCR, the dosage was increased to 4.5 mg of vinblastine (VCR) per kg body weight per day. Six (6) months of survival time was recorded.

- Table 74 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VCR	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	17	30	27	30
2 months	30	4	22	11	30
3 months	30	0	17	0	30
4 months	30	0	4	0	30

5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 75

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	21.4 ± 2.6	98.7 ± 16.6
2NY	all animals dead	all animals dead
2VCR	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	22.2 ± 2.5	not applicable

13. Uterine Cancer

5 The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.605 cells. Two mouse models of human uterine cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

10

Tumor Growth in Mouse Model

Murine transplantable uterine cancer type CRL-1622 closely resembles the human uterine cancer pathology. The uterine cancer cell line CRL-1622 can be ordered from the American Type Culture Collection.

15

The animals used to generate the uterine cancer cells for the experiments were female 615 mice (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 4 to 6 months, having an average body weight of about 20 to 22 gram. About 1.2×10^7 viable tumor cells of the uterine cancer cell line CRL-1622 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

20

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that group MMC was used in lie of group CTX, wherein the mice were injected subcutaneously with 0.8 mg of mitomycin C (MMC) per kg body weight per day.

5

Table 76

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	18.9 ± 1.7	1.56 ± 0.54
NY	17.6 ± 2.3	4.43 ± 2.45
MMC	18.3 ± 3.1	3.56 ± 2.32
CK1	17.4 ± 3.7	4.82 ± 2.46
CK2	19.6 ± 2.6	not applicable

Survival Time in Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2MMC, the dosage was increased to 1.2 mg of mitomycin C (MMC) per kg body weight per day. Six (6) months of survival time was recorded.

Table 77 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2MMC	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	21	28	23	30
2 months	30	11	26	12	30
3 months	30	2	18	3	30
4 months	30	0	7	0	30
5 months	30	0	0	0	30

6 months	30	0	0	0	30
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Table 78

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.2 ± 2.5	78.7 ± 17.4
2NY	all animals dead	all animals dead
2MMC	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.4 ± 2.6	not applicable

Tumor Growth in Kun Ming Mouse Model

The animals used to generate the uterine cancer cells for the experiments were female kun ming mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 5 to 6 months, having an average body weight of about 20 to 22 gram. About 1.2×10^7 viable tumor cells of the uterine cancer cell line HTB-114 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that group VCR was used in lie of group CTX, wherein the mice were injected intravenously with 3 mg of vinblastine (VCR) per kg body weight per day.

Table 79

Group	mean weight of mice and standard deviation (g)	Mean weight of tumor nodules and standard deviation (g)
AY	19.6 ± 2.6	1.65 ± 0.67
NY	17.7 ± 2.7	4.33 ± 2.34
VCR	18.3 ± 3.3	4.36 ± 2.45

CK1	18.4 ± 3.4	4.32 ± 2.36
CK2	20.5 ± 2.5	not applicable

Survival Time in Kun Ming Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2VCR, the dosage was increased to 4.5 mg of vinblastine (VCR) per kg body weight per day. Six (6) months of survival time was recorded.

Table 80 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VCR	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	30	28	21	30
2 months	30	16	19	17	30
3 months	30	3	11	5	30
4 months	30	0	3	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 81

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	21.6 ± 2.7	103.5 ± 17.8
2NY	all animals dead	all animals dead
2VCR	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	22.7 ± 2.4	not applicable

14. Kidney Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.189 cells. A hamster model and the Wistar rat model of human kidney cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Hamster Model

The following example illustrates the benefit of a biological composition of the invention in a Syrian hamster model of human kidney cancer. The growth of the tumor in the hamsters was studied. Detailed description of the use of Syrian hamster to study kidney cancer can be found in, e.g., Liehr et al., 1991, Carcinogenesis 12:385-9, which is incorporated herein by reference in its entirety.

Complex cancer cells of kidney closely resembles the human kidney cancer pathology. See e.g., Modern Clinical Experimental Protocols, People's Republic of China Health Publishing House, 1997.

The animals used to generate the kidney cancer cells for the experiments were Syrian hamsters (obtainable from the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences, Beijing, China), an equal number of male and female, 5 to 6 months old, having an average body weight of 150 to 180 gram. The complex cancer cells of kidney was generated from neutered male Syrian hamsters which were induced by subcutaneous injection of 17- β -hydroxysteroid. About 2×10^6 viable tumor cells of the complex cancer cells of kidney (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that of the section Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that the dosages for groups AY, NY, CK1 and CK2 were 0.5 ml once per. In addition, group FUDR was used in lieu of group CTX, wherein the hamsters were injected subcutaneously with 0.6 mg of fluoruridine (FUDR) per kg body weight per day.

Table 82

Group	mean weight of hamsters and standard deviation (g)	Mean weight of tumor nodules and standard deviation (mg)
AY	122.5 ± 6.4	0.6 ± 0.4
NY	108.4 ± 4.4	3.3 ± 1.7
FUDR	116.7 ± 4.9	2.7 ± 1.2
CK1	106.6 ± 4.7	3.2 ± 1.8
CK2	123.7 ± 3.8	Not applicable

Survival Time in Hamster Model

- This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 83 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2FUDR	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	25	27	21	30
2 months	30	9	11	7	30
3 months	30	0	5	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 84

Group	mean weight of hamsters and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
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2AY	134.8 ± 5.6	187.3 ± 12.6
2NY	all animals dead	all animals dead
2FUDR	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	142.7 ± 7.9	not applicable

Tumor Growth in Wistar Rat Model

Complex cancer cells of kidney closely resembles the human kidney cancer pathology. See e.g., Modern Clinical Experimental Protocols, People's Republic of China Health Publishing House, 1997.

The animals used to generate the kidney cancer cells for the experiments were Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China) having an average body weight of about 180 to 200 gram. The complex cancer cells of kidney (obtainable from the Shanghai Medical University, Shanghai, China) were generated from a Wistar rat with kidney cancer that was induced by subcutaneous injection of bismaleimido-hexane (BHM). About 2.5×10^7 viable tumor cells of the complex cancer cells of kidney in about 0.4 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that of the section Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except that the dosages for groups AY, NY, CK1 and CK2 were 0.8 ml once per, and a group VCR was used in lieu of group TSPA, wherein the rats were injected intravenously with 3 mg of vinblastine (VCR) per kg body weight per day. The treatment continued for 30 days and the rats were scarified on the 31st day.

20

Table 85

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	197.4 ± 12.2	0.6 ± 0.4
NY	185.2 ± 12.7	4.4 ± 2.4
VCR	189.6 ± 2.5	3.7 ± 1.9

CK1	184.4 ± 12.9	4.3 ± 2.6
CK2	198.6 ± 12.3	not applicable

Survival Time in Wistar Rat Model

This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 86 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VCR	Group 2CK1	Group 2CK2
0 month	30	28	30	30	30
1 month	30	22	30	26	30
2 months	30	19	30	16	30
3 months	30	7	22	4	30
4 months	30	3	13	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 87

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	216 ± 12.4	257.5 ± 12.6
2NY	All animals dead	all animals dead
2VCR	All animals dead	all animals dead
2CK1	All animals dead	all animals dead
2CK2	221 ± 12.1	not applicable

15. Bladder Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.4 cells. Mouse model and Wistar rat model of bladder cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Mouse Model

Numerous animal studies have reported the use of murine transplantable bladder cancer animal models. Murine transplantable bladder cancer cell type N-butyl-N-(4-hydroxybutylnitrosamine (BBN) induced T₇₃₉ closely resembles the human bladder cancer pathology. Detailed description of the use of murine transplantable bladder cancer cell type BBN-induced T₇₃₉ can be found in, e.g., Li L. et al., 2002, Sheng Wu Hua Xue Yu Sheng 34:21-7, which is incorporated herein by reference in its entirety.

The BBN-induced T₇₃₉ cells (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) were obtained from a 12th generation cell line.

The animals used to receive the bladder cancer cells were T739 mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China) having an average body weight of about 18 to 20 gram. About 10⁶ viable tumor cells of the bladder cancer cells T₇₃₉ (in about 0.2 ml culture suspension) were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that group TSPA was used in lieu of group CTX, wherein the mice were injected intravenously with 1.5 mg of thiotepa (TSPA) per kg body weight per day.

Table 88

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	20.8 ± 2.9	1.3 ± 0.6*
NY	18.2 ± 2.1	4.2 ± 2.7

TSPA	19.5 ± 2.2	3.3 ± 2.1
CK1	18.4 ± 2.7	3.9 ± 2.4
CK2	21.3 ± 2.2	not applicable

Survival Time in Mouse Model

This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 89 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2TSPA	Group 2CK1	Group 2CK2
0 month	30	26	30	24	30
1 month	30	19	24	21	30
2 months	30	8	11	7	30
3 months	30	0	7	0	30
4 months	30	0	2	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 90

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	22.7 ± 2.7	143.3 ± 12
2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	22.5 ± 2.4	not applicable

Tumor Growth in Wistar Rat Model

Murine transplantable bladder cancer type N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) closely resembles the human bladder cancer pathology. Numerous animal studies have reported the use of murine transplantable bladder cancer animal models. Detailed description of the use of murine transplantable bladder cancer cell type N-butyl-N-(4-hydroxybutyl)nitrosamine can be found in, e.g., Lummen G. et al., 2002, Urol Res. 30:199-203, which is incorporated herein by reference in its entirety.

The animals used to generate the bladder cancer cells for the experiments are Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 8 weeks old, with an average body weight of about 180 to 200 gram. Under sterile conditions, about 10^6 viable tumor cells of the bladder cell line BBN (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that of the section Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except that the dosages for groups AY, NY, CK1 and CK2 were 0.3 ml once per, and a group VCR was used in lieu of group TSPA, wherein the rats were injected intravenously with 3 mg of vinblastine (VCR) per kg body weight per day. The treatment continued for 30 days and the rats were scarified on the 31st day.

Table 91

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	203.4 \pm 6.3	0.73 \pm 0.12*
NY	187.5 \pm 13.1	3.44 \pm 2.6
VCR	191.3 \pm 12.4	2.65 \pm 1.7
CK1	182.8 \pm 12.8	3.36 \pm 2.4
CK2	204.2 \pm 12.4	not applicable

Survival Time in Wistar Rat Model

This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 92 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VCR	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	30	30	30	30
2 months	30	24	30	22	30
3 months	30	13	30	17	30
4 months	30	0	30	8	30
5 months	30	0	28	0	30
6 months	30	0	26	0	30

5

Table 93

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	218 ± 12.2	37.5 ± 9.7
2NY	all animals dead	all animals dead
2VCR	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	209 ± 11.8	not applicable

16. Brain Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.501 cells. The Kun Ming mouse model and Wistar rat

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model of human brain cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Kun Ming Mouse Model

5 The kun ming murine brain cancer cell line B22 was used in the model which resembles human brain cancer pathology. See e.g., In Oncology Basic and Research Protocols (Ed. J. Kao), People's Health Publishing House, Beijing, China, 1998, p.78.

10 The animals used to generate the brain cancer cells for the experiments are male kun ming mice (obtainable from the Chinese Academy of Military Medicine Sciences, Beijing, China), 6 to 7 months, having an average body weight of about 20 to 22 gram. About 1.2×10^7 viable tumor cells of the kun ming murine brain cancer cell line B22 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected
15 subcutaneously into the animals.

 The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group BCNU was used in lieu of group CTX, wherein the mice were injected subcutaneously with 0.8 mg of chloroethylnitrosourea (BCNU) per kg body weight
20 per day.

Table 94

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	18.7 ± 1.9	0.65 ± 0.45
NY	17.5 ± 2.6	1.56 ± 0.54
BCNU	18.4 ± 3.4	1.02 ± 0.52
CK1	17.6 ± 3.4	1.82 ± 0.65
CK2	19.5 ± 2.4	not applicable

Survival Time in Kun Ming Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2BCNU, the dosage was increased to 1.2 mg of chloroethylnitrosourea (BCNU) per kg body weight per day. Six (6) months of survival time was recorded.

Table 95 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2BCNU	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	17	21	23	30
2 months	30	7	15	8	30
3 months	30	0	4	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 96

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.6 ± 2.6	88.6 ± 16.7
2NY	all animals dead	all animals dead
2BCNU	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.5 ± 2.7	not applicable

Tumor Growth in Wistar Rat Model

The Wistar King rat brain cancer cell line WKS-1 was used in the model which resembles human brain cancer pathology. See e.g., In Oncology Basic

and Research Protocols (Ed. J. Kao), People's Health Publishing House, Beijing, China, 1998, p.79.

The animals used to generate the brain cancer cells for the experiments are Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), both males and females, having an average body weight of about 180 to 200 gram. About 2×10^7 viable tumor cells of the Wistar King rat brain cancer type WKS-1 (obtainable from the Shanghai Medical University, Shanghai, China) in about 0.4 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that in the section of Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except that the dosages for groups AY, NY, CK1 and CK2 were 0.8 ml once per, and a group VCR was used in lieu of group TSPA, wherein the rats were injected intravenously with 3 mg of vinblastine (VCR) per kg body weight per day. The treatment continued for 30 days and the rats were scarified on the 31st day.

15

Table 97

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	196.6 \pm 9.6	0.76 \pm 0.38
NY	187.7 \pm 8.7	3.83 \pm 2.43
VCR	188.3 \pm 8.3	2.87 \pm 2.54
CK1	184.4 \pm 8.4	3.92 \pm 2.66
CK2	201.5 \pm 8.5	not applicable

Survival Time in Wistar Rat Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 1.2 ml and, for group 2VCR, the dosage was increased to 4.5 mg of vinblastine (VCR) per kg body weight per day. Six (6) months of survival time was recorded.

Table 98 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VCR	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	30	30	30	30
2 months	30	22	30	30	30
3 months	30	14	17	19	30
4 months	30	4	7	6	30
5 months	30	0	3	0	30
6 months	30	0	0	0	30

Table 99

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	212.6 ± 12.7	313.5 ± 24.6
2NY	all animals dead	all animals dead
2VCR	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	223.7 ± 12.4	not applicable

17. Lymphoma

- The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.562 cells. Two mouse models of lymphoma were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Inbred PBA Mouse Model

- A model based on inbred PBA mice which manifest a high incidence of tumor was used (Bailey et al., 1970, J Natl. Cancer Inst. 45(1):59-73). Mice six to

eight weeks old were treated by the method as described in Shiyan Dongwu Yu Zhongliu Yanjiu (Experimental Animal & Tumor Research), April 2000, by Chinese Medical Technology Publisher.

5 The mice that developed lymphoma spontaneously and that had been treated by the method as described above were divided into two series of five groups: one series received treatment for 30 days, the other series received treatment for 60 days. In any one experimental series, each treatment group has a total of 36 mice.

10 In the first series, the mice in group AY received 0.3 ml of the oral composition of the invention per day. The mice in group NY received 0.3 ml per day of a composition comprising at a similar concentration the same strain of yeast which had not been activated and conditioned. The mice in group CTX received subcutaneous injection of cyclophosphamide at a dose of 30 mg per kg body weight per day. The mice in group CK1 received physiological saline, while the mice in group CK2 received physiological saline but did not receive transplanted lymphoma cells. The experiment was carried out over a 30-day treatment period.

20 In the second series, the mice in group 2AY received 0.5 ml of the oral composition of the invention per day. The mice in group 2NY received 0.5 ml per day of a composition comprising the same strain of yeast which had not been activated and conditioned at a similar concentration. The mice in group 2CTX received subcutaneous injection of cyclophosphamide at a dose of 45 mg per kg body weight per day. The mice in group 2CK1 received physiological saline, while the mice in group 2CK2 received physiological saline but did not receive lymphoma cells. The experiment was carried out over a 60-day treatment period.

25 After the 30-day or 60-day treatment, the mice were fed a normal diet and allowed to live under the same environment. The health and survival of the mice were observed and compared over 180 days.

30 The results of the first series of five groups which received treatment for a total of 30 days are shown in Table 4. Each group consisted of 36 mice at the beginning of the experiment (3 repeats). The total number of surviving mice in each group were counted at the end of every month up to 6 months after treatment stopped.

Table 100

Days after treatment period	Group CK2 (saline, no lymphoma cells)	Group CK1 (saline only)	Group CTX (30 mg/kg cyclophosphamide ml per day)	Group AY (0.3 ml oral composition s per day)	Group NY (0.3 ml non-activated yeasts per day)
0	36	34	36	36	33
30	36	22	33	36	26
60	36	0	27	36	21
90	36	0	19	36	0
120	36	0	17	34	0
150	36	0	0	34	0
180	36	0	0	34	0

The results of the second series of five groups which received treatment for a total of 60 days are shown in Table 5. Each group consisted of 36 mice at the beginning of the experiment (3 repeats). The total number of surviving mice in each group were counted at the end of every month up to 6 months after treatment stopped.

Table 101

Days after treatment period	Group 2CK2 (saline, no lymphoma cells)	Group 2CK1 (saline only)	Group 2CTX (30 mg/kg cyclophosphamide ml per day)	Group 2AY (0.5 ml oral composition s per day)	Group 2NY (0.5 ml non-activated yeasts per day)
0	36	36	36	36	35
30	36	25	36	36	28
60	36	11	29	36	19
90	36	4	21	36	11
120	36	0	14	36	6
150	36	0	14	36	0

180	36	0	8	36	0
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The above results indicate that the lifespan of PBA mice with spontaneous lymphoma was prolonged as a result of receiving the oral composition of the invention at a dose of 0.3 ml per day or 0.5 ml per day for a 30-day or 60-day treatment period. The results also showed that in comparison to cyclophosphamide, the oral composition of the invention at a dose of 0.3 ml per day or 0.5 ml per day for a 30-day or 60-day treatment period prevented the recurrence of the lymphoma in the animals after treatment ceased.

Athymic Nude Mouse Model

A model based on transplantation of Burkitt's lymphoma cell in athymic nude mice was used (Gurtsevitch et al., 1988, Int J Cancer 15;41(1):87-95). The model was set up as described in Shiyang Dongwu Yu Zhongliu Yanjiu (Experimental Animal & Tumor Research), April 2000, by Chinese Medical Technology Publisher. Essentially, seven week old mice were injected intravenously each with 1×10^6 Burkitt's lymphoma cells.

Twenty-one days after the injection of lymphoma cells, the mice were divided into two series of five groups: one series received treatment for 30 days, the other series received treatment for 60 days. In any one experimental series, each treatment group has a total of 36 mice. The mice in group A received saline orally. The mice in group B received subcutaneous injection of cyclophosphamide at a dose of 30 mg per kg body weight per day. Group C received subcutaneous injection of cyclophosphamide at a dose of 45 mg per kg body weight per day. The mice in group D received 0.3 ml of the oral composition per day. group E received 0.5 ml of the oral composition per day.

After the 30-day or 60-day treatment, the mice were fed a normal diet and allowed to live under the same environment. The health and survival of the nude mice were observed and compared over 360 days.

The results of the first series of five groups which received treatment for a total of 30 days are shown in Table 7. Each group consisted of 36 mice at the beginning of the experiment (3 repeats). The total number of surviving mice in each group were counted at the end of every month up to 12 months after treatment stopped.

Table 102

Days after treatment period	Group A (0.5 saline per day)	Group B (30 mg/kg cyclophosphamide ml per day)	Group C (45 mg/kg cyclophosphamide ml per day)	Group D (0.3 ml oral compositions per day)	Group E (0.5 ml oral compositions per day)
0	36	36	35	36	36
30	36	33	35	35*	36
60	36	24	27	35	36
90	35*	14	21	35	36
120	35	5	17	35	36
150	35	3	11	35	36
180	35	1	4	35	36
210	35	0	2	35	36
240	35	0	1	35	36
270	35	0	0	35	34*
300	34*	0	0	35	34
330	34	0	0	35	34
360	34	0	0	35	34

* The mice died of other causes unrelated to the lymphoma.

The results of the second series of five groups which received treatment for a total of 60 days are shown in Table 6. Each group consisted of 36 mice at the beginning of the experiment (3 repeats). The total number of surviving mice in each group were counted at the end of every month up to 12 months after treatment stopped.

Table 103

Days after treatment period	Group A (0.5 saline per day)	Group B (30 mg/kg cyclophosphamide ml per day)	Group C (45 mg/kg cyclophosphamide ml per day)	Group D (0.3 ml oral composition s per day)	Group E (0.5 ml oral composition s per day)
0	36	35	34	35*	36
30	36	35	34	35	36
60	36	33	34	35	36
90	36	24	27	35	36
120	36	19	19	35	36
150	36	9	12	35	36
180	36	5	7	35	36
210	36	2	3	35	36
240	36	0	0	35	36
270	36	0	0	35	35*
300	36	0	0	35	35
330	34*	0	0	35	35
360	34	0	0	35	35

* The mice died of other causes unrelated to the lymphoma.

The above results indicate that the lifespan of athymic nude mice transplanted with Burkitt's lymphoma cells was prolonged as a result of receiving the oral composition of the invention at a dose of 0.3 ml per day or 0.5 ml per day for a 30-day or 60-day treatment period. The results also showed that in comparison to cyclophosphamide, the oral composition of the invention at a dose of 0.3 ml per day or 0.5 ml per day for a 30-day or 60-day treatment period prevented the recurrence of the lymphoma in the animals after treatment ceased.

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18. Leukemia

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces*

cerevisiae Hansen strain AS2.11 cells. Two mouse models of human leukemia were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment. Leukemia cell line HL-60 was also used to study the benefit of the biological composition in regulating the changes in cell cycles.

5 Tumor Growth in SWI Mouse Model

Murine transplantable leukemia cell line L6565 closely resembles the human leukemia pathology. See, *e.g.*, Lab Animal and Animal Experiment Techniques, China Chinese Medicine Publisher, 1997, which is incorporated herein by reference in its entirety.

10 The animals used to generate the leukemia cells for the experiments were SWI mice (obtainable from the Chinese Academy of Military Medicine Science, Beijing, China), both males and females, with an average body weight of about 18 to 22 gram. About 10^6 viable tumor cells of the leukemia cell line L6565 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, Cancer) in
15 about 0.2 ml spleen cell culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that 21 days of consecutive treatment was performed in lieu of the 30 day treatment
20 regime.

Table 104

Group	number of leukemia cells and standard deviation (cells/ml)
AY	0.0 ± 0.0
NY	$2,930 \pm 798$ (all animals died between 9 to 13 days after injection of tumor; the number of tumor cells were measured at the time of death)
CTX	933 ± 241
CK1	$2,860 \pm 831$ (all animals died between 9 to 15 days after injection of tumor; the number of tumor cells were measured at the time of death)
CK2	0.0 ± 0.0

Survival Time in SWI Mouse Model

This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 105 Number of live animals remaining in the groups after 21 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	19	28	17	30
1 month	30	0	19	0	30
2 months	30	0	3	0	30
3 months	30	0	0	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

5

Table 106

Group	number of leukemia cells and standard deviation (cells/ml)
2AY	0.0 ± 0.0
2NY	all animals dead
2CTX	all animals dead
2CK1	all animals dead
2CK2	0.0 ± 0.0

Tumor Growth in T₇₃₉ Mouse Model

Detailed description of the use of T₇₃₉ mice to study cancer can be found in, e.g., Niu Q. *et al.*, 2001, *Zhonghua Zhong Liu Za Zhi.* 23(5):382-4, and Zheng F. *et al.*, 2000, *Zhonghua Jie He He Hu Xi Za Zhi.* 23(1):34-6, each of which is incorporated herein by reference in its entirety.

The animals used to generate the leukemia cells for the experiments were T₇₃₉ mouse (obtainable from the Chinese Academy of Military Medicine Sciences, Beijing, China), both males and females, with an average body weight of

about 18 to 22 gram. About 10^6 viable tumor cells of the leukemia cell line T₇₃₉ in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that 21 days of consecutive treatment was performed in lieu of the 30 day treatment regime.

Table 107

Group	number of leukemia cells and standard deviation (cells/ml)
AY	0.0 ± 0.0
NY	$2,032 \pm 643$ (all animals died between 10 to 15 days after injection of tumor; the number of tumor cells were measured at the time of death)
CTX	957 ± 132
CK1	$2,087 \pm 712$ (all animals died between 11 to 17 days after injection of tumor; the number of tumor cells were measured at the time of death)
CK2	0.0 ± 0.0

10 Survival Time in T₇₃₉ Mouse Model

This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 108 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	0	28	0	30
1 month	30	0	19	0	30
2 months	30	0	3	0	30
3 months	30	0	0	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30

6 months	30	0	0	0	30
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Table 109

Group	number of leukemia cells and standard deviation (cells/ml)
2AY	0.0 ± 0.0
2NY	all animals dead
2CTX	all animals dead
2CK1	all animals dead
2CK2	0.0 ± 0.0

Cell Regulation

5 The following example illustrates the benefit of a biological composition of the invention in regulating cell changes in a leukemia cell line HL-60. The leukemia cell line HL-60 is commonly used to establish the efficacy of a treatment. See, *e.g.*, Modern Pharmacology Experimental Protocols, Peking Union Medical College Publisher, 1998.

10 Flow cytometry was used to study the changes in cell cycles. Flow cytometry measures the number of cells in different cell cycles by analyzing the amount of DNA in a single cell. A fluorescent dye, propidium iodide (PI) intercalates with DNA and emits yellow and red regions fluorescence in response to 488 nm excitation. Cells in the G1 and G0 cell cycle phases will contain a 2n amount of
15 DNA. Cells in the G2 and M phases will contain a 4n amount of DNA, while cells in S phase will have a DNA content between 2n and 4n (as the DNA has only partially been replicated). In particular, flow cytometry can be used to determine the percentage of live cells in each phase of the cell cycle and the percentage of dead cells. This information, coupled with the doubling time, allows one to calculate the
20 length of each cell cycle phase. More detailed description of the methodologies and applications of flow cytometry can be found in Darzynkiewicz *et al.*, 2001, *Clin Lab Med.* 21(4):857-73, which is incorporated by reference herein in its entirety.

 Activated HL-60 leukemia cells (obtainable from the Cancer Institute, Chinese Academy of Military Medical Sciences, Beijing, China) were cultured in
25 RPMI-1640 suspension and then divided into 3 groups of 10^9 cells per group. The

three groups of HL-60 cells were quintuplicated. Detailed description of the cultivation of human leukemia HL-60 cells can be found in, *e.g.*, Saether A.K. *et al.*, 1991, *Eur J Cell Biol.* 55(2):346-51, which is incorporated by reference herein in its entirety.

5 In the experimental group AY, the biological composition was filtered through 0.22 μm filter. The activated and conditioned yeast cells (about 5 μm in width and 10 μm in length) were collected and added to 10^9 HL-60 leukemia cells per ml to make 5% final concentration by weight. In the comparative group NY, the untreated yeast cells were filtered through 0.22 μm filter. The untreated yeast cells
10 (about 5 μm in width and 10 μm in length) were collected and added to 10^9 HL-60 leukemia cells per ml to make 5% final concentration by weight. In the control group CK, 10^9 HL-60 leukemia cells per ml was used.

Each group of cells were observed after culturing for 24 hours and 72 hours. Afterwards, the cells were stained with propidium iodide and the percentage of
15 cells at different cell cycle stages were observed using flow cytometry. Detailed description of the use of flow cytometry to study different cell cycle stages can be found in Hagsawa S. *et al.*, *Med Electron Microsc.* 1999;32(3):167-174).

Tables 10 and 11 show the percentages of live leukemia cells in each cell cycle and the percentages of dead leukemia cells in the treatment and control
20 groups after culturing for 24 hours and 72 hours, respectively.

Table 110 Percentages of live leukemia cells and dead leukemia cells after 24 hours

Cell Cycle Stage	AY (% of cells)	NY (% of cells)	CK (% of cells)
G ₀ -G ₁	28.9%	33.2%	33.0%
S	50.8%	44.4%	44.3%
G ₂ -M	20.3%	22.4%	22.6%
Programmed Cell Death	24.7%	3.2%	3.1%

After culturing the HL-60 leukemia cells with the activated and conditioned yeast cells for 24 hours (group AY), 24.7% of the HL-60 leukemia cells
25 were dead and 75.3% of the leukemia cells were alive (100% - 24.7%). Out of the total number of live leukemia cells, 28.9% were in G₀-G₁ stage; 50.8% were in S

stage; and 20.3% were in G₂-M stage, *i.e.*, 28.9% + 50.8% + 20.3% = 100% (total live leukemia cells).

In contrast, after culturing the HL-60 leukemia cells with untreated yeast cells for 24 hours (group NY), 3.2% of the leukemia cells were dead and 96.8% of the leukemia cells were alive (100% - 3.2%). Out of the total number of live leukemia cells, 33.2% were in G₀-G₁ stage; 44.4% were in S stage; and 22.4% were in G₂-M stage, *i.e.*, 33.2% + 44.4% + 22.4% = 100% (total live leukemia cells).

Similarly, after culturing the HL-60 leukemia cells alone for 24 hours (group CK), only 3.1% of the leukemia cells were dead and 96.9% of the leukemia cells were alive (100% - 3.1%). Out of the total number of live leukemia cells, 33.0% were in G₀-G₁ stage; 44.3% were in S stage; and 22.6% were in G₂-M stage, *i.e.*, 33.0% + 44.3% + 22.6% = 99.9% (total live leukemia cells).

After 24 hours, the percentage of programmed cell death is significantly greater (8x) when the HL-60 leukemia cells were co-incubated with the activated and conditioned yeast cells of the present invention (24.7% in group AY) than when the HL-60 leukemia cells were co-incubated with the untreated yeast cells (3.2% in group NY) or cultured alone (3.1% in group CK). At 24 hours, co-incubation with the activated and conditioned yeast cells of the present invention have already begun to reduce the number of live leukemia cells. A significant number of live leukemia cells in all three groups were at S stage, *i.e.*, when DNA replication occurs (synthesis phase).

Table 111 Percentages of live leukemia cells and dead leukemia cells after 72 hours

Cell Cycle Stage	AY (% of cells)	NY (% of cells)	CK (% of cells)
G ₀ -G ₁	56.2%	41.6%	41.3%
S	36.1%	40.2%	40.7%
G ₂ -M	7.7%	18.2%	18.0%
Programmed Cell Death	85.2%	11.6%	11.3%

After culturing the HL-60 leukemia cells with the activated and conditioned yeast cells for 72 hours (group AY), 85.2% of the leukemia cells were dead and 14.8% of the leukemia cells were alive (100% - 85.2%). Out of the total

number of live leukemia cells, 56.2% were in G₀-G₁ stage; 36.1% were in S stage; and 7.7% were in G₂-M stage, *i.e.*, $56.2\% + 36.1\% + 7.7\% = 100\%$ (total live leukemia cells).

In contrast, after culturing the HL-60 leukemia cells with untreated yeast cells for 72 hours (group NY), 11.6% of the leukemia cells were dead and 88.4% of the leukemia cells were alive (100% - 11.6%). Out of the total number of live leukemia cells, 41.6% were in G₀-G₁ stage; 40.2% were in S stage; and 18.2% were in G₂-M stage, *i.e.*, $41.6\% + 40.2\% + 18.2\% = 100\%$ (total live leukemia cells).

Similarly, after culturing the HL-60 leukemia cells alone for 72 hours (group CK), only 11.3% of the leukemia cells were dead and 88.7% of the leukemia cells were alive (100% - 11.3%). Out of the total number of live leukemia cells, 41.3% were in G₀-G₁ stage; 40.7% were in S stage; and 18.0% were in G₂-M stage, *i.e.*, $41.3\% + 40.7\% + 18.0\% = 100.0\%$ (total live leukemia cells).

After 72 hours, the percentage of programmed cell death is still significantly greater (8x) when the HL-60 leukemia cells were co-incubated with the activated and conditioned yeast cells of the present invention (85.2% in group AY) than when the HL-60 leukemia cells were co-incubated with the untreated yeast cells (11.6% in group NY) or cultured alone (11.3% in group CK). The percentage of programmed cell deaths in group AY increased more than three-folds from 24.7% to 85.2%, suggesting that the activated and conditioned yeast cells of the present invention are extremely effective in reducing the number of live leukemia cells. Further, more than half of the live leukemia cells in group AY at 72 hours (56.2%) are now in G₀-G₁ stage, *i.e.*, a temporary or permanent resting period where the cell has reached an end stage of development and will no longer divide.

25

The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

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All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

1. A biological composition comprising activated yeast cells, wherein said yeast cells are prepared by a method comprising at least two steps selected from the group consisting of:

2. A biological composition comprising activated and conditioned yeast cells, wherein the yeast cells are prepared by a method comprising activating the yeast cells, said activating comprising at least two steps selected from the group consisting of:

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9,907 to 12,285 MHz and a field strength in the range of 230 to 430 mV/cm;

(d) culturing the yeast cells in a fourth electromagnetic field having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 220 to 450 mV/cm; and

(e) culturing the yeast cells in a fifth electromagnetic field having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm,

and conditioning the activated yeast cells, said conditioning

comprising at least one step selected from the group consisting of:

(f) culturing the yeast cells in a liquid medium comprising wild hawthorn juice and gastric juice of a mammal in a sixth electromagnetic field having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 220 to 450 mV/cm; and

(g) culturing the yeast cells in a liquid medium comprising wild hawthorn juice and gastric juice of a mammal in a seventh electromagnetic field having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm.

3. A biological composition comprising activated and conditioned yeast cells, wherein the activated and conditioned yeast cells of claim 2 are subjected to at least one period of culturing in a liquid medium comprising wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice, and in the presence of in any order:

(h) an eighth electromagnetic field or series of electromagnetic fields having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 170 to 450 mV/cm; and

(i) a ninth electromagnetic field or series of electromagnetic fields having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 120 to 450 mV/cm.

4. The biological composition of claim 1, 2, or 3, wherein the activated yeast cells are at a concentration of about 10^6 to 10^9 cells per ml.

5. The biological composition of claim 1, 2, or 3, wherein the activated yeast cells are dried and at a concentration of about 10^7 to 10^{10} cells per gram.

5 6. A pharmaceutical composition comprising the activated and conditioned yeast cells of claim 1, 2 or 3, and a pharmaceutical acceptable carrier.

7. A dietary supplement comprising the activated and conditioned yeast cells of claim 1, 2 or 3, and one or more ingredients selected from the group consisting of vitamins, herbs, herbal extracts, minerals, amino acids, metal chelates,
10 plant extracts, coloring agents, flavor enhancers and preservatives.

8. A nutritional composition comprising the activated and conditioned yeast cells of claim 1, 2 or 3, and a food product selected from the group consisting of a fruit juice-based beverage, a tea-based beverage, a dairy product, a soybean product, and a rice product.

15 9. A method for preparing a biological composition comprising activated yeast cells, said method comprising at least two steps selected from the group consisting of:

- (a) culturing yeast cells in a first electromagnetic field having a frequency in the range of 7,821 to 10,170 MHz and a field strength in the range of 200 to 350 mV/cm;
20
- (b) culturing the yeast cells in a second electromagnetic field having a frequency in the range of 7,993 to 11,530 MHz and a field strength in the range of 190 to 330 mV/cm;
- (c) culturing the yeast cells in a third electromagnetic field
25 electromagnetic fields having a frequency in the range of 9,907 to 12,285 MHz and a field strength in the range of 230 to 430 mV/cm;
- (d) culturing the yeast cells in a fourth electromagnetic field having a frequency in the range of 11,141 to 12,842 MHz and
30 a field strength in the range of 220 to 450 mV/cm; and

- (e) culturing the yeast cells in a fifth electromagnetic field having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm.

10. A method for preparing a biological composition comprising activated
5 and conditioned yeast cells, said method comprising activating the yeast cells, said activating comprising at least two steps selected from the group consisting of:

- (a) culturing yeast cells in a first electromagnetic field having a frequency in the range of 7,821 to 10,170 MHz and a field strength in the range of 200 to 350 mV/cm;
- 10 (b) culturing the yeast cells in a second electromagnetic field having a frequency in the range of 7,993 to 11,530 MHz and a field strength in the range of 190 to 330 mV/cm;
- (c) culturing the yeast cells in a third electromagnetic field
15 electromagnetic fields having a frequency in the range of 9,907 to 12,285 MHz and a field strength in the range of 230 to 430 mV/cm;
- (d) culturing the yeast cells in a fourth electromagnetic field having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 220 to 450 mV/cm; and
- 20 (e) culturing the yeast cells in a fifth electromagnetic field having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm,

and conditioning the activated yeast cells, said conditioning
comprising at least one step selected from the group consisting of:

- 25 (f) culturing the yeast cells in a liquid medium comprising wild hawthorn juice and gastric juice of a mammal in a sixth electromagnetic field having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 230 to 440 mV/cm; and
- 30 (g) culturing the yeast cells in a liquid medium comprising wild hawthorn juice and gastric juice of a mammal in a seventh electromagnetic field having a frequency in the range of

12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm.

11. A method of making a biological composition comprising activated and conditioned yeast cells, said method comprising culturing the activated and conditioned yeast cells prepared by the method of claim 18 in a liquid medium comprising wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice, and in the presence of in any order:

- (h) an eighth electromagnetic field or series of electromagnetic fields having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 170 to 450 mV/cm; and
- (i) a ninth electromagnetic field or series of electromagnetic fields having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 120 to 450 mV/cm.

12. The method of claim 10 or 11 further comprising after the culturing step drying the activated and conditioned yeast cells.

13. The method of claim 12, wherein the drying step comprises:

- (a) drying at a temperature not exceeding 65°C for a period of time such that the yeast cells become dormant; and
- (b) drying at a temperature not exceeding 70°C for a period of time to reduce the moisture content to below 5%.

14. A method for retarding the growth of cancer cells in a mammal comprising administering orally to the mammal an effective amount of the biological composition of claim 1, 2 or 3.

15. A method for prolonging the time of survival of a mammal with cancer comprising administering orally to the mammal an effective amount of the biological composition of claim 1, 2 or 3.

16. The biological composition of claim 1, 2 or 3 for use as a medicament.

17. Use of the biological composition of claim 1, 2 or 3 for the manufacture of a medicament for the treatment of cancer.

1/2

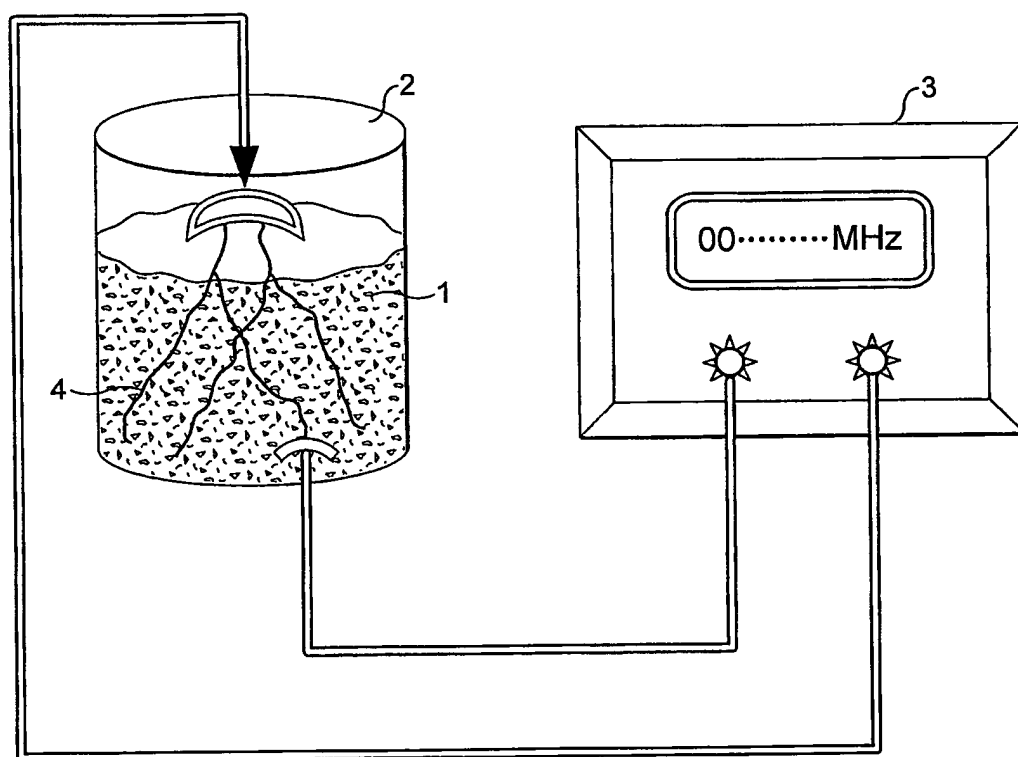


FIG. 1

2 / 2

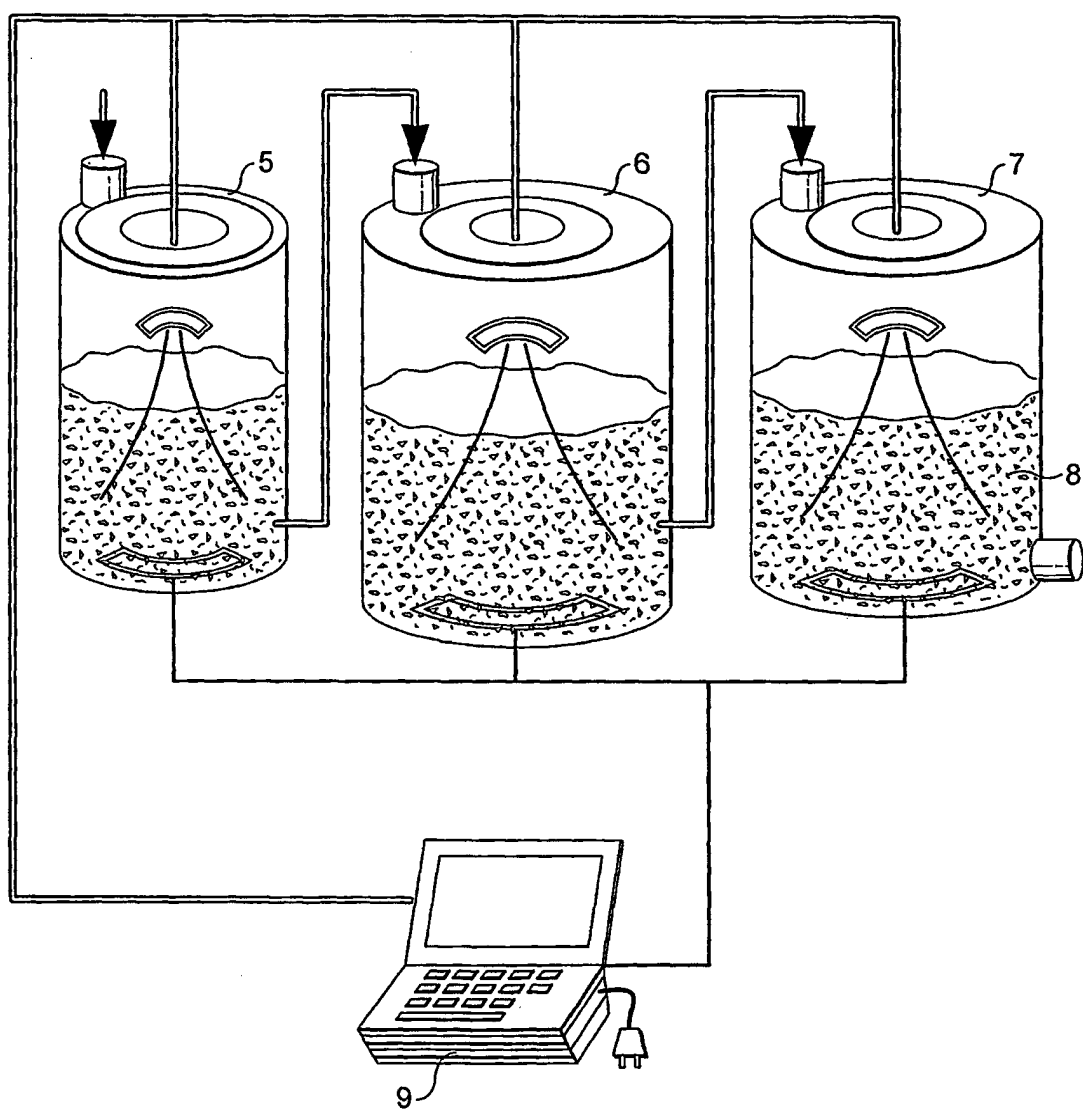


FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/002466

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N13/00 C12N1/16 C12N1/18 A61K35/72 A61K41/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, MEDLINE, BIOSIS, EMBASE, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 200267 Derwent Publications Ltd., London, GB; Class B04, AN 2002-627548 XP002259470 & WO 02/062983 A1 (SIX FOREST BIO-SCI INST LTD) 15 August 2002 (2002-08-15)	1-8, 14-17
A	abstract	9-13
X	WO 02/070683 A (CHEUNG LING YUK ; ULTRA BIOTECH LTD (GB)) 12 September 2002 (2002-09-12)	1-8
A	claims 14-19	9-17
	----- -/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

B document member of the same patent family

Date of the actual completion of the international search

12 August 2004

Date of mailing of the international search report

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PONNE C T ET AL: "Interaction of electromagnetic energy with biological material--relation to food processing" RADIATION PHYSICS AND CHEMISTRY, ELSEVIER SCIENCE PUBLISHERS BV., AMSTERDAM, NL, vol. 45, no. 4, 1 April 1995 (1995-04-01), pages 591-607, XP004051598 ISSN: 0969-806X page 597; table 2 page 598 - page 599; table 3	1-8
A	page 602; table 4	9-17
X	BINNINGER D M ET AL: "Effects of 60Hz AC magnetic fields on gene expression following exposure over multiple cell generations using Saccharomyces cerevisiae" BIOELECTROCHEMISTRY AND BIOENERGETICS, XX, XX, vol. 43, no. 1, 1997, pages 83-89, XP002223047 ISSN: 0302-4598	1-8
A	page 83, right-hand column, line 13 - line 15 page 84, left-hand column, line 1 - line 4	9-17
P,X	EP 1 375 652 A (ULTRA BIOTECH LTD) 2 January 2004 (2004-01-02)	1-8,16
P,A	claims 1-30	9-15,17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2004/002466

Box II Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 14 and 15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 02062983	A1	15-08-2002	NONE
WO 02070683	A	12-09-2002	US 6391617 B1 21-05-2002
			US 2002123127 A1 05-09-2002
			US 2002123130 A1 05-09-2002
			US 6391618 B1 21-05-2002
			US 2002123128 A1 05-09-2002
			US 2002123129 A1 05-09-2002
			US 6391619 B1 21-05-2002
			US 2002123126 A1 05-09-2002
			EP 1364001 A2 26-11-2003
			EP 1368463 A2 10-12-2003
			WO 02070682 A2 12-09-2002
			WO 02070683 A2 12-09-2002
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Published:

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*For two-letter codes and other abbreviations, refer to the "Guid-
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WO 02/070683 A2

(54) Title: BIOLOGICAL COMPOSITIONS FOR SOLID WASTE TREATMENT

(57) Abstract: The present invention relates to biological compositions useful for the treatment of solid waste. The biological compositions of the invention comprises a plurality of yeast cells which is capable of suppression of growth of pathogenic microorganisms, breakdown of undesirable chemicals, such as antibiotics, insecticides and waste chemicals, and reducing the odor of organic waste matters. The yeast cells of the invention are produced by culturing the plurality of yeast cells under activation conditions in the presence of a series of electromagnetic fields. The invention also relates to methods for manufacturing the treatment composition.

BIOLOGICAL COMPOSITIONS FOR SOLID WASTE TREATMENT

1. FIELD OF THE INVENTION

5 The invention relates to biological compositions that comprise yeasts for the treatment of solid waste. The yeasts in the compositions of the invention have been stimulated to perform a variety of functions including degradation of chemicals, reduction of odor and suppression of microorganisms. The invention also relates to methods for manufacturing the biological compositions, and methods for using the biological
10 compositions to treat waste.

2. BACKGROUND OF THE INVENTION

 Large amounts of solid waste are generated daily by industrial and agricultural activities, and by municipalities. If the waste is not treated properly, it can
15 cause severe and long-lasting damage to the environment. In 1995-1996, 208 million tons of municipal solid waste were generated in the United States. Of the municipal solid waste generated, 56 million tons (27 percent) were recovered by recycling or composting, 33.5 million tons (16 percent) were combusted at high temperatures, and 118.5 million tons (57 percent) were landfilled.

20 Municipal waste may be treated instead of landfilled. One type of municipal waste treatment involves high temperature burning of the waste in an incinerator. This combustion of municipal waste significantly reduces its volume. The ash from municipal waste combustion must be properly managed to prevent the environmental damage from any potential hazardous constituents. Also, emissions from the incinerator's smoke stack
25 must be within acceptable regulatory levels.

 In the agricultural area, despite the importance of mineral fertilizers in providing mankind with abundant agricultural products, the harm done to the environment has been recognized in recent years. Mineral fertilizers may incurred damages to soils. For example, most nitrogen fertilizers may acidify soils, thereby adversely affecting the growth
30 of plants and other soil organisms. Extensive use of chemical nitrogen fertilizers may also inhibit the activity of natural nitrogen fixing microorganisms, thereby decreasing the natural fertility of soils. The long term use of mineral fertilizers may also cause severe environmental pollution. For example, the loss of nitrogen and phosphate fertilizers due to leaching and soil erosion has led to contamination of soil and ground water, and
35 eutrophication of surface water.

Another type of agricultural waste is manure which, if not stored or disposed of properly, can pose health and environmental threats. For example, it can cause air pollution, i.e., odor and dust; and contamination of surface and ground water with excess nutrients, organic matter, salts, and pathogens. For example, manure contains pathogenic
5 microorganisms, such as *Escherichia coli*, *Salmonella spp.*, and *Shigella spp.*

Overall, cleaning up pollution as a result of a poor waste management strategy has been a complicated and difficult task. The cost for such a task is also astronomical. Thus, there is a need for inexpensive and effective methods to handle the waste generated by the myriad of human activities.

10 The use of biological compositions in pollution control has been proposed for many situations. Biological fertilizers utilizing microorganisms have been proposed as alternatives to mineral fertilizers. Naturally occurring nitrogen fixing microorganisms including bacteria, such as *Rhizobium*, *Azotobacter*, and *Azospirillum*, (See for example, U. S. Patent No. 5,071,462) and fungi, such as *Aspergillus flavus-oryzae*, (See, for example, U.
15 S. Patent No. 4,670,037) have been utilized in biological fertilizers. Naturally occurring microorganisms capable of solubilizing phosphate rock ore or other insoluble phosphates into soluble phosphates have also been utilized in biological fertilizers either separately (e.g., U. S. Patent No. 5,912,398) or in combination with nitrogen fixing microorganisms (e.g., U. S. Patent No. 5,484,464). An approach based on recombinant DNA techniques has
20 been developed to create more effective nitrogen fixing, phosphorus decomposing, and potassium decomposing bacterial strains for use in a biological fertilizer, see, for example, U.S. Patent No. 5,578,486; PCT publication WO 95/09814; Chinese patent publication: CN 1081662A; CN 1082016A; CN 1082017A; CN 1103060A; and CN 1109595A.

Citation of documents herein is not intended as an admission that any of the
25 documents cited herein is pertinent prior art, or an admission that the cited documents are considered material to the patentability of the claims of the present application. All statements as to the date or representations as to the contents of these documents are based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of these documents.

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3. SUMMARY OF THE INVENTION

The present invention relates to biological compositions useful for the treatment of solid waste. The biological compositions of the invention comprises a plurality of yeast cells which is capable of suppression of growth of pathogenic microorganisms,
35

breakdown of undesirable chemicals, such as antibiotics, insecticides and waste chemicals, and reducing the odor of organic waste matters.

In various embodiments, the present invention uses yeasts that are commercially available and/or accessible to the public, such as but not limited to
5 *Saccharomyces cerevisiae*. The yeast cells of the invention are produced by culturing the plurality of yeast cells under activation conditions in the presence of a series of electromagnetic fields, such that the yeast cells become highly efficient in performing certain metabolic functions. Accordingly, the invention also relates to methods for manufacturing the treatment composition comprising culturing the yeast cells under
10 activation conditions, mixing various yeast cell cultures of the present invention, followed by drying the yeast cells and packing the final product.

In particular, the invention encompasses methods for treatment of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the antibiotics in the solid waste, wherein said yeast cells are prepared by
15 culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having specific ranges of frequencies and field strengths. The antibiotics that can be degraded by the yeast cells of invention include are not limited to penicillin, chlortetracycline, oxytetracycline, doxycycline, tetracycline, streptomycin, kanamycin, erythromycin, spiramycin, bacitracin, colistin, chloramphenicol, cephalothin, neomycin and
20 novobiocin.

The invention also encompass methods for treatment of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields
25 having specific ranges of frequencies and field strengths. The undesirable chemicals that can be degraded by the yeast cells of the invention include but are not limited to toluene, ethylbenzene, trichlorophenols, dimethylbenzenes, benzaldehyde, propylaldehyde, nenanthaldehyde, dichlorobenzenes, acetophenone, arsanilic acid, roxarsone, furazolidonum, decoquinate, trichlorophonum, dinitomide, dichlorvos, momocrotophos,
30 dimethoate, DDT and toxaphene. The undesirable chemicals also include organic and inorganic salts such as ammonium compounds, nitrites or nitrates, and phosphates.

The invention further encompasses methods for reducing the odor of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of odorous molecules in the solid waste, wherein said yeast cells
35 are prepared by culturing the yeast cells in an electromagnetic field or a series of

electromagnetic fields having specific ranges of frequencies and field strengths. The odorous molecules include but are not limited to hydrogen sulfide, ammonia, indole, skatol, acetic acid, methylamine, and p-cresol.

The invention further encompasses methods for suppressing the growth of pathogenic bacteria in solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to suppress the growth of pathogenic bacteria in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having specific ranges of frequencies and field strengths. The pathogenic bacteria are selected from the group consisting of *Staphylococcus aureus*, *Diplococcus pneumoniae*, *Bacillus anthracis*, *Mycobacterium tuberculosis*, *Salmonella* species, *E. coli*, *Vibrio* species, *Shigella* species, *Clostridium botulinum*, and *Bacillus aerogenes capsulatus*.

The methods of the invention can be carried out using combinations of the yeast cells in the treatment of solid waste. A biological composition of the invention is added to the solid waste, said biological composition comprising at least one of the following yeast cell components (a) a first yeast cell component comprising a plurality of yeast cells that degrade antibiotics in solid waste; (b) a second yeast cell component comprising a plurality of yeast cells that degrade undesirable chemicals in solid waste; (c) a third yeast cell component comprising a plurality of yeast cells that reduce the amount of odorous molecules in solid waste; and (d) a fourth yeast cell component comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in the solid waste. The amount of time for the treatment can be determined empirically by monitoring the change in levels of the antibiotics, undesirable chemicals, pathogenic bacteria, and malodorous molecules in the solid waste, and can range from several hours, several days, and up to two or more weeks.

The invention further include methods for using the biological compositions of the present invention for the management, storage, processing, recycling or disposal of solid waste.

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4. BRIEF DESCRIPTION OF FIGURES

Fig. 1 Activation of yeast cells. 1 yeast cell culture; 2 container; 3 electromagnetic field source.

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Fig. 2. Adaptation of yeast cells to a soil type. 4 input electrode; 5 container; 6 electrode; 7 yeast cell culture; 8 electromagnetic field source; 9 temperature controller.

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5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides biological compositions that comprise yeast cells. The present invention also provides methods for manufacturing the biological compositions as well as methods for using the biological compositions.

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The biological compositions of the invention are useful for the treatment of solid waste so as to reduce the health risk and its impact on the environment that are often associated with its storage, transportation, processing, recycling and/or disposal. The use of such compositions may lower the overall cost of managing solid waste for a community, a business, or a farm, and make feasible the recycling of certain types of solid waste. As used
15 herein, treatment of a solid waste refers to a process which changes the physical, chemical, or biological character(s) of the solid waste that make the solid waste less objectionable while it is being stored, transported, recycled, handled, or less of a health threat or an environmental threat, than if the solid waste is not treated. The treatment generally renders the waste less hazardous, or makes the solid waste safer to transport, store, handle or
20 recycle.

According to the invention, the biological compositions comprise a plurality of yeast cell components. Each yeast cell component is a population of yeast cells which comprises a plurality of yeast cells that are capable of performing one or more desired functions falling within the following categories: (1) suppression of growth of pathogens,
25 (2) degradation of undesirable chemicals, or (3) reducing the odor of organic materials.

In one embodiment, a biological composition of the invention comprises at least one yeast cell component that can perform a function in one of the three categories. In preferred embodiments, the biological compositions of the invention comprises yeast cell components that provide functions in all three categories. Thus, the preferred biological
30 fertilizer compositions comprise at least three different yeast cell components. It will be understood that different alternative formulations of yeast cell components are contemplated.

As used herein, the term "solid waste" broadly refers to any kind of material that is discarded because it has served its purpose or it is a by-product that is of no use, and
35 that includes physiological waste excreted by humans and animals. The sources of solid

waste include residential, commercial, agricultural and industrial activities. Non-industrial and non-agricultural solid waste such as trash or garbage collected from urban areas, contains discarded food materials or materials used in food preparation, and other assorted dry materials, such as paper, fabric, or plastics. Especially in residential areas and
5 commercial areas which have restaurants and hotels, the predominant type of solid waste, herein referred to as "garbage", comprises mainly decomposable food wastes. Garbage which supports growth of pathogenic organisms and becomes malodorous due to decay, can be efficiently treated by the biological compositions of the invention. The type of solid waste that lends itself to treatment by the biological compositions of the invention has the
10 characteristic of a high organic content.

Another type of solid waste that can be treated by the compositions of the invention is sludge. The term "sludge" as used herein broadly encompasses any solid matter that has settled out of suspension in the course of sewage storage and/or treatment, for example but not limited to, residues in a waste lagoon, residues in an urban sewage
15 treatment plant, or sewage concentrate. The term "sludge" also include semi-solid matters, and mixtures of effluent and sediments. The term thus encompasses sludge having a wide range of viscosity, density, and water content, as well as sludge which has been partially processed or stabilized. Depending on the source, sludge may contain a variety of undesirable chemicals that may have an adverse impact on the environment if it is not
20 properly treated. Sludge is malodorous, and supports growth of pathogenic organisms.

The biological compositions of the invention can also treat waste products produced as a result of agricultural activities. Typically, the waste is produced by animals in operations such as but not limited to ranches, farms, slaughterhouses, and markets. The continuous production and accumulation of large amounts of animal excrement creates a
25 malodorous environment, and poses a health risk to humans and livestock due to the presence of pathogenic microorganisms. Agricultural waste can also contain undesirable chemicals, such as antibiotic feed additives, chemical fertilizers, pesticides, and herbicides, that may pollute the environment if the waste is not managed properly.

The term "animal manure" as used herein broadly encompasses organic
30 material that comprises the feces and urine of feedlot animals with or without accompanying litter such as straw, hay, or bedding, that is traditionally used to fertilize land. Poultry manure includes but are not limited to manure produced by domesticated birds, such as chicken, duck, turkey, goose, quail, squab, ostrich, and the like. Poultry manure include excrement or guano produced by non-domesticated bird species. Cattle manure as used
35 herein encompasses waste from domesticated ruminant mammals, such as dairy cows, or

beef cattle. The term "cattle manure" as used herein is not limited to just cattle but include other animals that graze, and that are kept primarily for their milk, meat, skin, hair, and pelts. Cattle manure includes but are not limited to manure produced by buffalos, bison, yaks, horses, donkeys, mules, sheep, goats, camels, and the like. Cattle manure also include
5 excrement produced by non-domesticated herds. The term "swine manure" as used herein includes but are not limited to manure produced by swines, hogs, pigs, and the like. Other agricultural waste include field crop residues, bagasse, waste from fruit and vegetable packing facilities, waste from animal product packing facilities which include animal carcasses.

10 In various embodiments, the biological compositions of the invention are particularly useful in treating garbage, sludge, and manure.

Municipal waste in many cases is temporarily stored at waste transfer stations. At the transfer station, waste is off-loaded from local collection routes and in some cases sorted according to type. The waste is then loaded onto larger trucks or rail cars for
15 transport to either a municipal waste treatment or disposal facility. Generally, depending on the source of the solid waste, glass, metal, wood, and other inorganic or non-decomposable items are separated from waste of a high organic content through sorting and separating operations. These can be carried out by methods well known in the recycling / garbage disposal industry, such as mechanically, using differences in such physical characteristics of
20 the solid waste as size, and density. Shredding or pulverizing can reduce the size of the waste articles to fine particles, resulting in a uniform mass of material which can be more easily handled, e.g., mixing or transport. Due to the variation of constituents in manure, sludge, or garbage, it may be desirable to subject a sample of a batch of waste material to analysis to determine the amount and type of pathogenic organisms and undesirable
25 chemicals present in the batch.

While the following terms are believed to have well-defined meanings in the art, the following are set forth to facilitate explanation of the invention.

As used herein, the phrase "suppressing the growth of pathogens" refers to a decrease or lack of increase in the number of pathogenic microorganisms present in a
30 sample of solid waste over a period of time, as a result of the presence of the yeast cells of the invention in the sample. It is to be understood that in the absence of the yeast cells, the number of pathogens in the sample would increase naturally. Many such microorganisms cause diseases in humans and animals, and may include bacteria such as *Escherichia* species, *Salmonella* species, *Shigella* species, *Mycobacterium* species, *Staphylococcus*
35 species, *Bacillus* species, *Streptococcus* species, and *Diplococcus* species.

As used herein, the phrase "degradation of undesirable chemicals" refers to biological or biochemical processes which result in the conversion of chemical compounds that are undesirable, e.g., environmental toxins, in solid waste to an inactive form, such as the breakdown of such compounds into lower molecular weight compounds. Antibiotics
5 are commonly present in manure and such compounds are not desired in a fertilizer made from manure because of the potential risk of ingestion by humans, for example, by eating vegetables grown using a fertilizer comprising contaminated organic material, and the possible spread of antibiotic resistance in the environment. Many antibiotics are added to animal feed to protect various farm animals, such as chicken, turkey, and swine, from
10 bacterial and parasitic diseases, and to promote growth. A significant amount of antibiotic feed additive is excreted by the animals, and thus accumulates in manure and sludge. Many kinds of antibiotics have been used in animal operations, such as but not limited to aminoglycosides, tetracyclines, beta-lactams, glycopeptides, and macrolides. Examples of antibiotics approved for use in farms in United States include but are not limited to,
15 bacitracin methylene disalicylate, bacitracin zinc, bambarmycins, oxytetracycline, chlortetracycline, penicillin, tylosin/sulfamethazine, roxarsone, nitrasone, monensin, lasalocid, carbodox, tiamulin, hygromycin B, nystatin, novobiocin, sulfadimethoxine, ormetoprim, lincomycin, fenbendazole, and virginiamycin. The presence and quantity of such antibiotics in a composition can be determined by any methods known in the art, for
20 example, high performance liquid chromatography (HPLC).

As used herein, the phrase "reducing the odor of organic materials" refers to a process which results in a lower concentration of one or more odorous compounds in the organic materials present in solid waste. Odorous compounds, such as but not limited to hydrogen sulfide, ammonia, indole, skatole (i.e, 3-methyl-1H-indole), p-cresol, and organic
25 acids, are known to contribute to the malodorous quality of solid waste. The concentration of such malodorous compounds in, for example, poultry manure or in a sample of air in contact with the manure can be determined by any method well known in the art, including but not limited to gas chromatography, or mass spectrometry. Odor is a perception of smell by an organism with olfactory organs. A reduction of the intensity of the odor associated
30 with solid waste can be determined subjectively. Various methods and techniques are known to measure the intensity of an odor. One subjective measurement of odor intensity is to measure the dilution necessary so that the odor is imperceptible or doubtful to a human or animal test panel. Alternatively, a recognition threshold may also be used which is a higher concentration at which the character of the odor is recognized. Any methods and

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techniques for objectively or subjectively determine the intensity of an odor can be used to monitor the performance of the compositions and methods of the invention.

The inventor discovered that, under certain culture conditions, yeasts can be activated to become highly efficient in certain metabolic functions which result in an ability of the activated yeasts to suppress growth of pathogens, degrade undesirable chemicals, or reduce the odor of organic materials.

According to the invention, a yeast cell component of the biological fertilizer composition is produced by culturing a plurality of yeast cells in an appropriate culture medium in the presence of an alternating electromagnetic field or multiple alternating electromagnetic fields in series over a period of time. The culturing process allows yeast spores to germinate, yeast cells to grow and divide, and can be performed as a batch process or a continuous process. As used herein, the terms "alternating electromagnetic field", "electromagnetic field" or "EM field" are synonymous. An electromagnetic field useful in the invention can be generated by various means well known in the art. A schematic illustration of exemplary setups are depicted respectively in Fig. 1. An electromagnetic field of a desired frequency and a desired field strength is generated by an electromagnetic wave source (3) which comprises one or more signal generators that are capable of generating electromagnetic waves, preferably sinusoidal waves, and preferably in the frequency range of 30 MHz - 3000 MHz. Such signal generators are well known in the art. Signal generators capable of generating signal with a narrower frequency range can also be used. If desirable, a signal amplifier can also be used to increase the output signal, and thus the strength of the EM field.

The electromagnetic field can be applied to the culture by a variety of means including placing the yeast cells in close proximity to a signal emitter connected to a source of electromagnetic waves. In one embodiment, the electromagnetic field is applied by signal emitters in the form of electrodes that are submerged in a culture of yeast cells (1). In a preferred embodiment, one of the electrodes is a metal plate, and the other electrode comprises a plurality of wires configured inside the container (2) so that the energy of the electromagnetic field can be evenly distributed in the culture. The number of electrode wires used depends on both the volume of the culture and the diameter of the wire. For example, for a culture having a volume of 5000 ml, one electrode wire having a diameter of between 0.1 to 1.2 mm can be used for each 100 ml of culture; for a culture having a volume greater than 1000 l, one electrode wire having a diameter of between 3 to 30 mm can be used for each 1000 l of culture. See Figure 1.

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Without being bound by any theory or mechanism, the inventor believes that the culture conditions activate and/or enhance the expression of a gene or a set of genes in a yeast cell such that the cell becomes more efficient in performing certain metabolic activities which lead to the respective desired results.

- 5 In various embodiments, yeasts of the genera of *Saccharomyces*, *Schizosaccharomyces*, *Sporobolomyces*, *Torulopsis*, *Trichosporon*, *Wickerhamia*, *Ashbya*, *Blastomyces*, *Candida*, *Citeromyces*, *Crebrothecium*, *Cryptococcus*, *Debaryomyces*, *Endomycopsis*, *Geotrichum*, *Hansenula*, *Kloeckera*, *Lipomyces*, *Pichia*, *Rhodospiridium*, and *Rhodotorula* can be used in the invention.
- 10 Non-limiting examples of yeast strains include *Saccharomyces cerevisiae* Hansen, ACCC2034, ACCC2035, ACCC2036, ACCC2037, ACCC2038, ACCC2039, ACCC2040, ACCC2041, ACCC2042, AS2.1, AS2.4, AS2.11, AS2.14, AS2.16, AS2.56, AS2.69, AS2.70, AS2.93, AS2.98, AS2.101, AS2.109, AS2.110, AS2.112, AS2.139, AS2.173, AS2.174, AS2.182, AS2.196, AS2.242, AS2.336, AS2.346, AS2.369, AS2.374, AS2.375, AS2.379, AS2.380, AS2.382, AS2.390, AS2.393, AS2.395, AS2.396, AS2.397, AS2.398, AS2.399, AS2.400, AS2.406, AS2.408, AS2.409, AS2.413, AS2.414, AS2.415, AS2.416, AS2.422, AS2.423, AS2.430, AS2.431, AS2.432, AS2.451, AS2.452, AS2.453, AS2.458, AS2.460, AS2.463, AS2.467, AS2.486, AS2.501, AS2.502, AS2.503, AS2.504, AS2.516, AS2.535, AS2.536, AS2.558, AS2.560, AS2.561, AS2.562, AS2.576, AS2.593, AS2.594, AS2.614, AS2.620, AS2.628, AS2.631, AS2.666, AS2.982, AS2.1190, AS2.1364, AS2.1396, IFFI 1001, IFFI 1002, IFFI 1005, IFFI 1006, IFFI 1008, IFFI 1009, IFFI 1010, IFFI 1012, IFFI 1021, IFFI 1027, IFFI 1037, IFFI 1042, IFFI 1043, IFFI 1045, IFFI 1048, IFFI 1049, IFFI 1050, IFFI 1052, IFFI 1059, IFFI 1060, IFFI 1063, IFFI 1202, IFFI 1203, IFFI 1206, IFFI 1209, IFFI 1210, IFFI 1211, IFFI 1212, IFFI 1213, IFFI 1215, IFFI 1220, IFFI 1221, IFFI 1224, IFFI 1247, IFFI 1248, IFFI 1251, IFFI 1270, IFFI 1277, IFFI 1287, IFFI 1289, IFFI 1290, IFFI 1291, IFFI 1292, IFFI 1293, IFFI 1297, IFFI 1300, IFFI 1301, IFFI 1302, IFFI 1307, IFFI 1308, IFFI 1309, IFFI 1310, IFFI 1311, IFFI 1331, IFFI 1335, IFFI 1336, IFFI 1337, IFFI 1338, IFFI 1339, IFFI 1340, IFFI 1345, IFFI 1348, IFFI 1396, IFFI 1397, IFFI 1399, IFFI 1411, IFFI 1413; *Saccharomyces cerevisiae* Hansen Var. ellipsoideus (Hansen) Dekker, ACCC2043, AS2.2, AS2.3, AS2.8, AS2.53, AS2.163, AS2.168, AS2.483, AS2.541, AS2.559, AS2.606, AS2.607, AS2.611, AS2.612; *Saccharomyces chevalieri* Guillermond, AS2.131, AS2.213; *Saccharomyces delbrueckii*, AS2.285; *Saccharomyces delbrueckii* Lindner var. mongolicus Lodder et van Rij, AS2.209, AS2.1157; *Saccharomyces exiguus* Hansen, AS2.349, AS2.1158; *Saccharomyces fermentati* (Saito) Lodder et van Rij, AS2.286, AS2.343; *Saccharomyces logos* van laer et
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- Denamur ex Jorgensen, AS2.156, AS2.327, AS2.335; *Saccharomyces mellis* Lodder et Kreger Van Rij, AS2.195; *Saccharomyces microellipsoides* Osterwalder, AS2.699; *Saccharomyces oviformis* Osterwalder, AS2.100; *Saccharomyces rosei* (Guilliermond) Lodder et kreger van Rij, AS2.287; *Saccharomyces rouxii* Boutroux, AS2.178, AS2.180, AS2.370, AS2.371; *Saccharomyces sake* Yabe, ACCC2045; *Candida arborea*, AS2.566; *Candida Krusei* (Castellani) Berkhout, AS2.1045; *Candida lambica* (Lindner et Genoud) van.Uden et Buckley, AS2.1182; *Candida lipolytica* (Harrison) Diddens et Lodder, AS2.1207, AS2.1216, AS2.1220, AS2.1379, AS2.1398, AS2.1399, AS2.1400; *Candida parapsilosis* (Ashford) Langeron et Talice, AS2.590; *Candida parapsilosis* (Ashford) et Talice Var. intermedia Van Rij et Verona, AS2.491; *Candida pulcherriman* (Lindner) Windisch, AS2.492; *Candida rugousa* (Anderson) Diddens et Loddeer, AS2.511, AS2.1367, AS2.1369, AS2.1372, AS2.1373, AS2.1377, AS2.1378, AS2.1384; *Candida tropicalis* (Castellani) Berkout, ACCC2004, ACCC2005, ACCC2006, AS2.164, AS2.402, AS2.564, AS2.565, AS2.567, AS2.568, AS2.617, AS2.1387; *Candida utilis* Henneberg Lodder et Kreger Van Rij, AS2.120, AS2.281, AS2.1180; *Crebrothecium ashbyii* (Guilliermond) Routein, AS2.481, AS2.482, AS2.1197; *Geotrichum candidum* Link, ACCC2016, AS2.361, AS2.498, AS2.616, AS2.1035, AS2.1062, AS2.1080, AS2.1132, AS2.1175, AS2.1183; *Hansenula anomala* (Hansen) H et P sydow, ACCC2018, AS2.294, AS2.295, AS2.296, AS2.297, AS2.298, AS2.299, AS2.300, AS2.302, AS2.338, AS2.339, AS2.340, AS2.341, AS2.470, AS2.592, AS2.641, AS2.642, AS2.635, AS2.782, AS2.794; *Hansenula arabitolgens* Fang, AS2.887; *Hansenula jadinii* Wickerham, ACCC2019; *Hansenula saturnus* (Klocker) H et P sydow, ACCC2020; *Hansenula schneeggii* (Weber) Dekker, AS2.304; *Hansenula subpelliculosa* Bedford, AS2.738, AS2.740, AS2.760, AS2.761, AS2.770, AS2.783, AS2.790, AS2.798, AS2.866; *Kloeckera apiculata* (Reess emend. Klocker) Janke, ACCC2021, ACCC2022, ACCC2023, AS2.197, AS2.496, AS2.711, AS2.714; *Lipomyces starkeyi* Lodder et van Rij, ACCC2024, AS2.1390; *Pichia farinosa* (Lindner) Hansen, ACCC2025, ACCC2026, AS2.86, AS2.87, AS2.705, AS2.803; *Pichia membranaefaciens* Hansen, ACCC2027, AS2.89, AS2.661, AS2.1039; *Rhodospiridium toruloides* Banno, ACCC2028; *Rhodotorula glutinis* (Fresenius) Harrison, ACCC2029, AS2.280, ACCC2030, AS2.102, AS2.107, AS2.278, AS2.499, AS2.694, AS2.703, AS2.704, AS2.1146; *Rhodotorula minuta* (Saito) Harrison, AS2.277; *Rhodotorula rubar* (Demme) Lodder, ACCC2031, AS2.21, AS2.22, AS2.103, AS2.105, AS2.108, AS2.140, AS2.166, AS2.167, AS2.272, AS2.279, AS2.282; *Saccharomyces carlsbergensis* Hansen, ACCC2032, ACCC2033, AS2.113, AS2.116, AS2.118, AS2.121, AS2.132, AS2.162, AS2.189, AS2.200, AS2.216, AS2.265, AS2.377, AS2.417, AS2.420,

- AS2.440, AS2.441, AS2.443, AS2.444, AS2.459, AS2.595, AS2.605, AS2.638, AS2.742, AS2.745, AS2.748, AS2.1042; *Saccharomyces uvarum* Beijer, IFFI 1023, IFFI 1032, IFFI 1036, IFFI 1044, IFFI 1072, IFFI 1205, IFFI 1207; *Saccharomyces willianus* Saccardo, AS2.5, AS2.7, AS2.119, AS2.152, AS2.293, AS2.381, AS2.392, AS2.434, AS2.614, AS2.1189; *Saccharomyces* sp., AS2.311; *Saccharomyces ludwigii* Hansen, ACCC2044, AS2.243, AS2.508; *Saccharomyces sinenses* Yue, AS2.1395; *Schizosaccharomyces octosporus* Beijerinck, ACCC 2046, AS2.1148; *Schizosaccharomyces pombe* Linder, ACCC2047, ACCC2048, AS2.248, AS2.249, AS2.255, AS2.257, AS2.259, AS2.260, AS2.274, AS2.994, AS2.1043, AS2.1149, AS2.1178, IFFI 1056; *Sporobolomyces roseus* Kluyver et van Niel, ACCC 2049, ACCC 2050, AS2.619, AS2.962, AS2.1036, ACCC2051, AS2.261, AS2.262; *Torulopsis candida* (Saito) Lodder, ACCC2052, AS2.270; *Torulopsis famta* (Harrison) Lodder et van Rij, ACCC2053, AS2.685; *Torulopsis globosa* (Olson et Hammer) Lodder et van Rij, ACCC2054, AS2.202; *Torulopsis inconspicua* Lodder et van Rij, AS2.75; *Trichosporon behrendii* Lodder et Kreger van Rij, ACCC2055, AS2.1193; *Trichosporon capitatum* Diddens et Lodder, ACCC2056, AS2.1385; *Trichosporon cutaneum* (de Beurm et al.) Ota, ACCC2057, AS2.25, AS2.570, AS2.571, AS2.1374; *Wickerhamia fluoresens* (Soneda) Soneda, ACCC2058, AS2.1388.

Certain yeast species that can be activated or induced according to the present invention and are included in the present invention are known to be pathogenic to human and/or other living organisms, for example, *Ashbya gossypii*; *Blastomyces dermatitidis*; *Candida albicans*; *Candida parakrusei*; *Candida tropicalis*; *Citeromyces matritensis*; *Crebrothecium ashbyii*; *Cryptococcus laurentii*; *Cryptococcus neoformans*; *Debaryomyces hansenii*; *Debaryomyces klockeri*; *Debaryomyces* sp.; *Endomycopsis fibuligera*. Under certain circumstances, it may be less preferable to use such pathogenic yeasts in the biological compositions of the invention, for example, if such use is in an open field, it may endanger the health of human and/or other living organisms.

Yeasts of the *Saccharomyces* genus are generally preferred. Among strains of *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* Hansen is a preferred strain. The most preferred strains of yeast are *Saccharomyces cerevisiae* strains having accession numbers AS2.504, AS2.558, AS2.413, AS2.397, AS2.69, AS2.109, AS2.607, AS2.516, AS2.561, AS2.422, AS2.393, AS2.631, AS2.982, AS2.560, AS2.467, AS2.415, AS2.375, AS2.628, AS2.1190, AS2.562, AS2.463, AS2.409, AS2.379, AS2.666, AS2.631, AS2.182, AS2.431, AS2.606, AS2.53, AS2.611, AS2.414, AS2.576, AS2.483, IFFI 1211, IFFI 1293, IFFI 1308, IFFI 1210, IFFI 1213, IFFI 1307, IFFI 1206, IFFI 1052, IFFI 1301, IFFI 1291, IFFI 1202, IFFI 1021, IFFI 1059, IFFI 1052, IFFI 1441, IFFI 1008, IFFI 1220, IFFI 1302,

and IFFI 1023 as deposited at the China General Microbiological Culture Collection Center (CGMCC).

Generally, yeast strains useful for the invention can be obtained from private or public laboratory cultures, or publically accessible culture deposits, such as the American
5 Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 and the China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture Collection of Microorganisms, Institute of Microbiology, Chinese Academy of Sciences, Haidian, P.O. Box 2714, Beijing, 100080, China.

Although it is preferred, the preparation of the yeast cell components of the
10 invention is not limited to starting with a pure strain of yeast. Each yeast cell component may be produced by culturing a mixture of yeast cells of different species or strains. The constituents of a yeast cell component can be determined by standard yeast identification techniques well known in the art.

The ability and efficiency of the activated yeasts to perform a desired
15 function before or after culturing under the conditions of the invention can readily be tested by methods known in the art. For example, HPLC or mass spectrometry can be used for detecting and analyzing various organic molecules in a sample of solid waste. Microbiological methods well known in the art can be used for detecting and counting the number of viable microorganisms and the total number of microorganisms in a sample.

20 When treating organic manure that has a relatively high bacteria count, the biological composition can be formulated to comprise largely yeast cells that suppress bacterial growth. When the biological composition is to be used to treat solid waste that is contaminated with undesirable chemicals, the biological composition can be formulated to comprise mostly yeast cells that degrade undesirable chemicals. Thus, the biological
25 compositions have utility in many types of situations encountered in municipal, commercial, agricultural, and industrial establishments. The invention can also be used domestically, especially in rural areas.

The biological compositions of the invention can be applied directly to the solid waste. As known to those skilled in the relevant art, many methods and appliances
30 may be used to mix the yeasts with the solid waste. In one embodiment, a culture broth of the yeasts of the present invention are added directly to the solid waste to be treated. In another embodiment, dried powders of the yeasts of the present invention are mixed with the solid waste to which water is added at a later time. The biological compositions may be applied to and mixed with the solid waste by spreaders, sprayers, and other mechanized
35 means which may be automated. The amount of biological composition used depends in

part on the circumstances and the type of solid waste, and can be determined empirically. However, to achieve an effective treatment, it is desired to use from about 300 to 600g dry weight (less than 10% moisture) of the biological composition per cubic meter of solid waste. The yeast cells are first mixed with water in the ratio of about 1000 g yeasts (dry weight) to about 30 liters, and then incubated for 12 to 24 hours, prior to application to the solid waste. The benefit of the treatment, e.g., reduction of odor or bacteria count, takes effect about 24 to 72 hours after the application. Although not necessary, the biological compositions of the invention can also be used in conjunction or in rotation with other types of deodorants, disinfectants, and detoxification agents.

Described respectively in Sections 5.1 - 5.4 are the yeast cell components used for degradation of antibiotics, pathogen suppression, degradation of undesirable chemicals, and reduction of odor. Methods for preparing each yeast cell components are described. Section 5.6 describes the manufacture of the biological compositions of the invention. In various embodiments of the invention, standard techniques for handling, transferring, and storing yeasts are used. Although it is not necessary, sterile conditions or clean environments are desirable when carrying out the manufacturing processes of the invention.

5.1. YEAST CELLS THAT DEGRADE ANTIBIOTICS

The present invention provides yeast cells that are capable of degrading antibiotics that are typically found in manures and sludge.

According to the invention, the ability of yeast cells to degrade antibiotics is activated or enhanced by culturing the yeast cells in the presence of an electromagnetic field in an appropriate culture medium. The resulting yeast cells can be used as a component in the biological solid waste treatment compositions of the invention.

The frequency of the electromagnetic field for activating or enhancing the ability of yeasts to degrade antibiotics can generally be found in the range of 70 MHz to 600 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their enhanced ability to decompose one or more types of antibiotics by methods well known in the art. Antibiotics degraded by the yeasts of the invention include but are not limited to molecules within the families of beta-lactams, tetracyclines, polypeptides, glycopeptides, aminoglycosides, and macrolides.

The method of the invention for making antibiotics-degrading yeasts is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose,

fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies
 5 between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate,
 10 and like ions. Non-limiting examples of nutrient inorganic salts are $(\text{NH}_4)_2\text{HPO}_4$, CaCO_3 , MgSO_4 , NaCl , and CaSO_4 .

Table 1: Composition for a culture medium for yeasts that degrade antibiotics

15	Medium Composition	Quantity
	Manure or sludge	8.0g, dry weight, >120 mesh
	NaCl	0.2g
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
20	$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	0.5g
	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.2g
	Peptone	1.5g
	K_2HPO_4	0.5g
25	Extract containing antibiotics (≥ 100 ug/ml)	600ml
	Autoclaved water	400ml

The extract containing antibiotics is prepared by dispersing and incubating
 30 500g of fresh waste, e.g., manures, sludge, in about 600ml of warm water (at 35-40°C) for 24 hours at 30-37°C, and filtering the fluid to remove particulate matters. If the extract contains only negligible amount of a particular antibiotics, an appropriate amount of the antibiotics can be added into the extract.

It should be noted that the composition of the media provided in Table 1 is
 35 not intended to be limiting. Various modifications of the culture medium may be made by

those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2 - 10^5 cell/ml, preferably 3×10^2 -
5 10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

10 The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 70.000 to 100.000 MHz and 410.000 to 620.000 MHz, preferably. The field strength of the EM field(s) is in the range of 40 to 250 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency
15 frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different
20 EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the
25 presence of the EM field or EM fields for a total of about 144 - 384 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of 8.5-85mV/cm, usually at about 50 mV/cm, is used. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased
30 to a higher level in the range of 150-250 mV, usually to about 200 mV. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m³, preferably 0.4 mol/m³. The oxygen level can be controlled by any
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conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C
5 to 4°C. The recovered yeast cells may also be dried and stored in powder form.

To determine the activity of the activated yeast cells towards an antibiotic compound, methods well known in the art, such as HPLC, can be used to measure the amounts of the antibiotic compound in a test sample at various time point and under different incubation conditions. For example, a sample containing known concentration of
10 an antibiotic (up to 100 mg per liter) is prepared. Then, 0.1 ml of activated and unactivated yeasts (at least 10^7 cells/ml) are added to the 100 liter samples containing the antibiotics, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the extracts are determined and compared by performing HPLC on the samples.

15 The method is generally applicable to many type of antibiotics. In specific embodiments, methods that are optimized for a particular type of antibiotics are described, *infra*.

Yeast cell component that decomposes penicillins

20 In a specific embodiment, a method for producing yeast cells that decompose penicillins, e.g., penicillin G and Cloxacillin, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 100.000 MHz, including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, and 100 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.399 were cultured at about
25 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 77 MHz at 48 mV/cm for 15 h; 83 MHz at 48 mV/cm for 15 h; 90 MHz at 48 mV/cm for 15 h; 96 MHz at 48 mV/cm for 15 h; 77 MHz at 200 mV/cm for 30 h; 83 MHz at 200 mV/cm for 30 h; 90 MHz at 200 mV/cm for 30 h; 96 MHz at 200 mV/cm for 30 h.

30 The activity of the activated yeast cells towards penicillin was determined by measuring the amounts of penicillin the activated yeast cells can degrade. Two 100 liter samples each containing a penicillin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control
35 was included which did not contain any yeast cells. After 24 hours, the amounts of

antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of penicillin in the sample with the activated yeast cells was reduced by more than 56.5%.

5 Yeast cell component that decomposes chlortetracycline

In a specific embodiment, a method for producing yeast cells that decompose chlortetracycline, e.g., aureomycin, chlortetracycline hydrochloridum, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 10 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.748 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 73 MHz at 48 mV/cm for 15 h; 88 MHz at 48 15 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards chlorotetracycline was determined by measuring the amounts of chlorotetracycline the activated yeast cells can degrade. Two 100 liter samples each containing a chlorotetracycline concentration of 20 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the two samples. Comparing to samples with 25 unactivated yeast cells, the amount of chlorotetracycline in the samples with the activated yeast cells was reduced by more than 62.3%.

Yeast cell component that decomposes oxytetracycline

30 In a specific embodiment, a method for producing yeast cells that decompose oxytetracycline, e.g., oxytetracycline hydrochloridum, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, Yeast cells of 35 *Saccharomyces cerevisiae* strain AS2.101 are cultured at about 25-30°C in a culture

medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 74 MHz at 48 mV/cm for 15 h; 88 MHz at 44 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 74 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

5 The activity of the activated yeast cells towards oxytetracycline was determined by measuring the amounts of oxytetracycline the activated yeast cells can degrade. Two 100 liter samples each containing an oxytetracycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and
10 incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with Comparing to samples unactivated yeast cells, the amount of oxytetracycline in the samples with the activated yeast cells was reduced by more than 65.5%.

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Yeast cell component that decomposes doxycycline

In a specific embodiment, a method for producing yeast cells that decompose doxycycline is provided. The frequencies of the EM field(s) used to activate the yeast cells
20 are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.417 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 71 MHz at 48 mV/cm for 15 h; 73 MHz at 48
25 mV/cm for 15 h; 77 MHz at 48 mV/cm for 15 h; 88 MHz at 48 mV/cm for 15 h; 71 MHz at 200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 77 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards doxycycline was determined by measuring the amounts of doxycycline the activated yeast cells can degrade. Two 100
30 liter samples each containing a doxycycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples are determined and compared by
35 performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the

amount of doxycycline in the samples with the activated yeast cells was reduced by more than 54.9%.

Yeast cell component that decomposes tetracycline

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In a specific embodiment, a method for producing yeast cells that decompose tetracycline is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.70 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 75 MHz at 48 mV/cm for 15 h; 82 MHz at 48 mV/cm for 15 h; 85 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 75 MHz at 200 mV/cm for 30 h; 82 MHz at 200 mV/cm for 30 h; 85 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards tetracycline was determined by measuring the amounts of tetracycline the activated yeast cells can degrade. Two 100 liter samples each containing a tetracycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples are determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of tetracycline in the samples with the activated yeast cells was reduced by more than 67.6%.

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Yeast cell component that decomposes streptomycin

In a specific embodiment, a method for producing yeast cells that decompose streptomycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.441 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 73 MHz at 48 mV/cm for 15 h; 80 MHz at 48 mV/cm for 15 h; 96 MHz at 48 mV/cm for 15 h; 70 MHz at

200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 80 MHz at 200 mV/cm for 30 h; 96 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards streptomycin was determined by measuring the amounts of streptomycin the activated yeast cells can degrade. Two 100 liter samples each containing a streptomycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of streptomycin in the samples with the activated yeast cells was reduced by more than 77.8%.

Yeast cell component that decomposes kanamycin

In a specific embodiment, a method for producing yeast cells that decompose kanamycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, east cells of *Saccharomyces cerevisiae* strain AS2.336 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 71 MHz at 48 mV/cm for 15 h; 78 MHz at 48 mV/cm for 15 h; 86 MHz at 48 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 71 MHz at 200 mV/cm for 30 h; 78 MHz at 200 mV/cm for 30 h; 86 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards kanamycin was determined by measuring the amounts of kanamycin the activated yeast cells can degrade. Two 100 liter samples each containing a kanamycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of kanamycin in the samples with the activated yeast cells was reduced by more than 68.7%.

Yeast cell component that decomposes erythromycin

In a specific embodiment, a method for producing yeast cells that decompose erythromycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.422 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 73 MHz at 48 mV/cm for 15 h; 79 MHz at 48 mV/cm for 15 h; 88 MHz at 48 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 73 MHz at 200 mV/cm for 30 h; 79 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards erythromycin was determined by measuring the amounts of erythromycin the activated yeast cells can degrade. Two 100 liter samples each containing a erythromycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of erythromycin in the samples with the activated yeast cells was reduced by more than 72.7%.

Yeast cell component that decomposes spiramycin

In a specific embodiment, a method for producing yeast cells that decompose spiramycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.620 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 77 MHz at 48 mV/cm for 15 h; 84 MHz at 48 mV/cm for 15 h; 93 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 77 MHz at 200 mV/cm for 30 h; 84 MHz at 200 mV/cm for 30 h; 93 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards spiramycin was determined by measuring the amounts of spiramycin the activated yeast cells can degrade. Two 100 liter samples each containing a spiramycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of spiramycin in the samples with the activated yeast cells was reduced by more than 66.8%.

Yeast cell component that decomposes bacitracin

In a specific embodiment, a method for producing yeast cells that decompose bacitracin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.486 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 75 MHz at 48 mV/cm for 15 h; 78 MHz at 48 mV/cm for 15 h; 81 MHz at 48 mV/cm for 15 h; 95 MHz at 48 mV/cm for 15 h; 75 MHz at 200 mV/cm for 30 h; 78 MHz at 200 mV/cm for 30 h; 81 MHz at 200 mV/cm for 30 h; 95 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards bacitracin was determined by measuring the amounts of bacitracin the activated yeast cells can degrade. Two 100 liter samples each containing a bacitracin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of bacitracin in the samples with the activated yeast cells was reduced by more than 71.6%.

Yeast cell component that decomposes colistin

In a specific embodiment, a method for producing yeast cells that decompose colistin or colistin sulfate is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.611 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 433 MHz at 85 mV/cm for 12 h; 440 MHz at 85 mV/cm for 12 h; 446 MHz at 85 mV/cm for 12 h; 457 MHz at 85 mV/cm for 12 h; 433 MHz at 204 mV/cm for 24 h; 440 MHz at 204 mV/cm for 24 h; 446 MHz at 204 mV/cm for 24 h; 457 MHz at 204 mV/cm for 24 h.

The activity of the activated yeast cells towards colistin was determined by measuring the amounts of colistin the activated yeast cells can degrade. Two 100 liter samples each containing a colistin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells: After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of colistin in the samples with the activated yeast cells was reduced by more than 71.6%.

Yeast cell component that decomposes chloramphenicol

In a specific embodiment, a method for producing yeast cells that decompose chloramphenicol and salts such as chloromycetin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.371 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 419 MHz at 85 mV/cm for 12 hr; 425 MHz at 85 mV/cm for 12 h; 433 MHz at 85 mV/cm for 12 h; 462 MHz at 85 mV/cm for 12 h; 419 MHz at 183 mV/cm for 24 h; 425 MHz at 183 mV/cm for 24 h; 433 MHz at 183 mV/cm for 24 h; 462 MHz at 183 mV/cm for 24 h.

The activity of the activated yeast cells towards chloramphenicol was determined by measuring the amounts of chloramphenicol the activated yeast cells can degrade. Two 100 liter samples each containing a chloramphenicol concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of chloramphenicol in the samples with the activated yeast cells was reduced by more than 58.6%.

Yeast cell component that decomposes cephalosporins

In a specific embodiment, a method for producing yeast cells that decompose cephalosporins, e.g., cephalothin, cephaloridine, cephaloglyin, cephalolexin, and cephaloline, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.559 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 434 MHz at 85 mV/cm for 12 h, 441 MHz at 85 mV/cm for 12 h, 450 MHz at 85 mV/cm for 12 h, 458 MHz at 85 mV/cm for 12 h; 434 MHz at 198 mV/cm for 24 h, 441 MHz at 198 mV/cm for 24 h, 450 MHz at 198 mV/cm for 24 h, 458 MHz at 198 mV/cm for 24 h.

The activity of the activated yeast cells towards cephalosporins was determined by measuring the amounts of cephalothin the activated yeast cells can degrade. Two 100 liter samples each containing a cephalothin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of cephalothins in the samples with the activated yeast cells was reduced by more than 75.5%.

Yeast cell component that decomposes neomycin

In a specific embodiment, a method for producing yeast cells that decompose neomycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 550.000 to 620.000 MHz, including but not limited to 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, and 575 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.182 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 557 MHz at 85 mV/cm for 12 h, 564 MHz at 85 mV/cm for 12 h, 568 MHz at 85 mV/cm for 12 h, 574 MHz at 85 mV/cm for 12 h; 557 MHz at 231 mV/cm for 24 h, 564 MHz at 231 mV/cm for 24 h, 568 MHz at 231 mV/cm for 24 h, 574 MHz at 231 mV/cm for 24 h.

The activity of the activated yeast cells towards neomycin was determined by measuring the amounts of neomycin the activated yeast cells can degrade. Two 100 liter samples each containing a neomycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of neomycin in the samples with the activated yeast cells was reduced by more than 67.7%.

Yeast cell component that decomposes novobiocin

In a specific embodiment, a method for producing yeast cells that decompose novobiocin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 550.000 to 620.000 MHz, including but not limited to 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, and 610 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.112 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 594 MHz at 85 mV/cm for 12 h, 599 MHz at 85 mV/cm for 12 h, 602 MHz at 85 mV/cm for 12 h, 608 MHz at 85 mV/cm for 12 h; 594 MHz at 231 mV/cm for 24 h, 599 MHz at 231 mV/cm for 24 h, 602 MHz at 231 mV/cm for 24 h, 608 MHz at 231 mV/cm for 24 h.

The activity of the activated yeast cells towards novobiocin was determined by measuring the amounts of novobiocin the activated yeast cells can degrade. Two 100 liter samples each containing a novobiocin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of novobiocin in the samples with the activated yeast cells was reduced by more than 69.5%.

5.2. YEAST CELL COMPONENTS THAT DECOMPOSE UNDESIRABLE CHEMICALS

The present invention further provides yeast cells that are capable of degrading chemicals that are typically found in solid waste.

According to the invention, the ability of yeast cells to degrade undesirable chemicals is activated or enhanced by culturing the yeast cells in the presence of an electromagnetic field in an appropriate culture medium. The resulting yeast cells can be used as a component in the biological waste treatment compositions of the invention.

The frequency of the electromagnetic field for activating or enhancing the ability of yeasts to degrade undesirable chemicals can generally be found in the range of 30 to 280 MHz, 410 to 440 MHz, 660 to 690 MHz, 1400 to 1435 MHz, and 1980 to 2210 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their enhanced ability to decompose one or more types of chemicals by methods well known in the art. Undesirable chemicals degraded by the yeasts of the invention include but are not limited to herbicides, pesticides, and fertilizer-related pollutants.

The method of the invention for making chemical-degrading yeasts is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5%

and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(\text{NH}_4)_2\text{HPO}_4$, CaCO_3 , MgSO_4 , NaCl , and CaSO_4 .

Table 2: Composition for a culture medium for yeasts that degrade chemicals

Medium Composition	Quantity
Manure or sludge	8.0g, dry weight, >120 mesh
NaCl	0.2g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	0.5g
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.2g
Peptone	1.5g
K_2HPO_4	0.5g
Extract containing chemicals (≥ 100 ug/ml)	600ml
Autoclaved water	400ml

The extract for the culture medium is prepared by incubating 500g of fresh waste, e.g., manures, sludge, and/or garbage, in about 600ml of warm water (at 35-40°C) for 24 hours at 30-37°C, and filtering the fluid to remove particulate matters. If the extract contains low amount of the particular chemicals, an appropriate amount of the chemical can be added into the extract.

It should be noted that the composition of the media provided in Table 2 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2 - 10^5 cell/ml, preferably 3×10^2 - 10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series

of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can
5 each have a frequency in the ranges of 30.000 to 100.000, 70.000 to 280.000, 410.000 to 430.000, 660.000 to 680.000 and 1980.000 to 2210.000 MHz. The field strength of the EM field(s) is in the range of 40 to 250 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated
10 ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

15 Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 90 - 480 hours.

20 For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of about 8 to about 300 mV/cm is used. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is
25 between 0.025 to 0.8 mol/m³, preferably 0.4 mol/m³. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C
30 to 4°C. The recovered yeast cells may also be dried and stored in powder.

To determine the activity of the activated yeast cells towards a chemical compound, methods well known in the art, such as HPLC, can be used to measure the amounts of the compound in a test sample at various time point and under different incubation conditions. For example, a sample containing a known concentration of a
35 chemical compound (up to 100 mg per liter) is prepared. Then, 0.1 ml of activated and

unactivated yeasts (at least 10^7 cells/ml) were added to the 100 liter samples containing the compound, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of compound remaining in the extracts are determined and compared by performing HPLC on the samples.

- 5 The method is generally applicable to many classes of chemicals. In specific embodiments, method that are optimized for a particular class of chemicals are described, infra.

Yeast cell component that decomposes aromatic compounds

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In specific embodiment, method for producing yeast cells that decompose trichlorophenol, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 30.000 to 100.000 MHz, or preferably 52 to 98 MHz including but not limited to 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 15 94, 96, and 98 MHz. Yeast cells of *Saccharomyces cerevisiae* strain IFFI1411 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 6 EM fields in the order stated: 82 MHz at 82 mv/cm for 25 h; 90 MHz at 82 mv/cm for 25 h; 98 MHz at 82mv/cm for 25 h; 82 MHz at 274 mv/cm for 32 h; 90 MHz at 274 mv/cm for 32 h; 98 MHz at 274 mv/cm for 25 h.

- 20 The activity of the activated yeast cells towards trichlorophenol was determined by measuring the amounts of the compound the activated yeast cells can degrade. Two 100 liter samples each containing the compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and 25 incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of the compound remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of tripchlorophenol in the samples with the activated yeast cells was reduced by more than 56.4%.

- 30 In another specific embodiment, method for producing yeast cells that decompose toluene or ethylbenzene, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 52.000 to 98.000 MHz, including but not limited to 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.56 were 35 cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a

series of 4 EM fields in the order stated: 76 MHz at 89 mV/cm for 20 h; 80 MHz at 89 mV/cm for 200 h; 86 MHz at 89 mV/cm for 20 h; and 96 MHz at 89 mV/cm for 20 h.

The activity of the activated yeast cells towards toluene or ethylbenzene was determined by measuring the amounts of the compounds the activated yeast cells can degrade. Two 100 liter samples each containing the compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of the compound remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of toluene in the samples with the activated yeast cells was reduced by more than 74.3%.

In another specific embodiment of the invention, a method for producing yeast cells that decompose dimethylbenzene compounds, e.g., p-xylene, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 30.000 to 50.000 MHz, or 70.000 to 98.000 MHz including but not limited to 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.420 are cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 72 MHz at 93 mV/cm for 20 h; 80 MHz at 93 mV/cm for 20 h; 88 MHz at 93 mV/cm for 20 h; and 98 MHz at 93 mV/cm for 20 h.

The activity of the activated yeast cells towards dimethylbenzene compounds was determined by measuring the amounts of dimethylbenzene compounds the activated yeast cells can degrade. Two 100 liter samples each containing a dimethylbenzene compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of p-xylene remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of p-xylene in the samples with the activated yeast cells was reduced by more than 66.6%.

Yeast cell component that decomposes aldehyde compounds

In another specific embodiment of the invention, a method for producing yeast cells that decompose benzaldehyde and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 133-151 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, or 151 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.374 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 130 mV/cm for 30 h; 86 MHz at 130 mV/cm for 30 h; 94 MHz at 130 mV/cm for 30 h; 96 MHz at 130 mV/cm for 30 h.

The activity of the activated yeast cells towards benzaldehyde was determined by measuring the amounts of benzaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing benzaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of benzaldehyde remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of benzaldehyde in the samples with the activated yeast cells was reduced by more than 63.6%.

In yet another specific embodiment, a method for producing yeast cells that decompose propylaldehyde and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 145-162 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, and 162 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.414 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 103 mV/cm for 20 h; 88 MHz at 103 mV/cm for 20 h; 96 MHz at 103 mV/cm for 20 h; 98 MHz at 103 mV/cm for 30 h.

The activity of the activated yeast cells towards propylaldehyde was determined by measuring the amounts of propylaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing propylaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast

cells. After 24 hours, the amounts of propylaldehyde remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of propylaldehyde in the samples with the activated yeast cells was reduced by more than 73.8%.

5 In yet another specific embodiment, a method for producing yeast cells that decompose nenanthaldehyde compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 MHz or 100.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.503 were cultured at about 25-
10 30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 81 MHz at 90 mV/cm for 12 h, 85 MHz at 90 mV/cm for 12 h, 89 MHz at 90 mV/cm for 12 h, 94 MHz at 90 mV/cm for 12 h, 81 MHz at 157 mV/cm for 24 h, 85 MHz at 157 mV/cm for 24 h, 89 MHz at 157 mV/cm for 24 h, 94 MHz at 157 mV/cm for 24 h.

15 The activity of the activated yeast cells towards nenanthaldehyde was determined by measuring the amounts of nenanthaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing nenanthaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and
20 incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of nenanthaldehyde remaining in the samples were determined and compared by performing HPLC on the two samples. Comparing to samples with unactivated yeast cells, the amount of nenanthaldehyde in the samples with the activated yeast cells was reduced by more than 81.3% in 24 hours.

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Yeast cell component that decomposes halogen-substituted benzene compounds

In a specific embodiment, method for producing yeast cells that decompose halogen-substituted benzene compounds, e.g., m-dichlorobenzene, is provided. The
30 frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 163.000 to 183.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, and 183 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.483 were cultured at about 25-30°C in a culture
35 medium as described in Table 2 in the presence of a series of 4 EM fields in the order

stated: 72 MHz at 107 mV/cm for 20 h; 80 MHz at 107 mV/cm for 10 h; 90 MHz at 107 mV/cm for 30 h; 94 MHz at 107 mV/cm for 40 h.

The activity of the activated yeast cells towards dichlorobenzene was determined by measuring the amounts of dichlorobenzene the activated yeast cells can
5 degrade. Two 100 liter samples each containing dichlorobenzene concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24
10 hours, the amounts of dichlorobenzene remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dichlorobenzene in the samples with the activated yeast cells was reduced by more than 64.6%.

Yeast cell component that decomposes acetophenone and related compounds

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In yet another embodiment, a method for producing yeast cells that decompose acetophenone and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 175.000 to 191.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92,
20 94, 96, 97, 98, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, and 191 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.265 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 124 mV/cm for 20 h; 82 MHz at 124 mV/cm for 30 h; 90 MHz at 124 mV/cm for 40 h; 98 MHz at 124 mV/cm for 20 h.

25

The activity of the activated yeast cells towards acetophenone was determined by measuring the amounts of acetophenone the activated yeast cells can degrade. Two 100 liter samples each containing acetophenone at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and
30 incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of acetophenone remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of acetophenone compounds in the samples with the activated yeast cells was reduced by more than 75.5%.

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Yeast cell component that decomposes arsanilic acid and related compounds

In yet another embodiment, a method for producing yeast cells that decompose arsanilic acid and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 183.000 to 205.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, and 205 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.745 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 133 mV/cm for 30 h; 88 MHz at 133 mV/cm for 40 h; 92 MHz at 133 mV/cm for 30 h; 96MHz at 133 mV/cm for 30 h.

The activity of the activated yeast cells towards arsanilic acid was determined by measuring the amounts of arsanilic acid compounds the activated yeast cells can degrade. Two 100 liter samples each containing an arsanilic acid at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of arsanilic acid antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of arsanilic acid in the samples with the activated yeast cells was reduced by more than 75.5%.

Yeast cell component that decomposes roxarsone and related compounds

In another specific embodiment, a method for producing yeast cells that decompose roxarsone and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 114.000 to 128.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, and 128 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.173 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 110 mV/cm for 10 h; 92 MHz at 110 mV/cm for 10h; 78 MHz at 213 mV/cm for 30 h; 92 MHz at 213 mV/cm for 30 h.

The activity of the activated yeast cells towards roxarsone was determined by measuring the amounts of roxarsone the activated yeast cells can degrade. Two 100 liter samples each containing roxarsone concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of roxarsone remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of roxarsone in the samples with the activated yeast cells was reduced by more than 67.9%.

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Yeast cell component that decomposes furazolidonum compounds

In yet another specific embodiment, a method for producing yeast cells that decompose furazolidonum and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 200.000 to 220.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, and 220 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.397 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 74 MHz at 98 mV/cm for 30 h; 76 MHz at 98 mV/cm for 20 h; 86 MHz at 98 mV/cm for 30 h; 94 MHz at 98 mV/cm for 30 h.

The activity of the activated yeast cells towards furazolidonum was determined by measuring the amounts of furazolidonum the activated yeast cells can degrade. Two 100 liter samples each containing furazolidonum concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of furazolidonum remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of furazolidonum in the samples with the activated yeast cells was reduced by more than 81.4%.

Yeast cell component that decomposes Decoquinat

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In yet another specific embodiment, a method for producing yeast cells that decompose decoquinatone and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 213.000 to 229.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, and 229 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.452 were cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 112 mV/cm for 30 h; 82 MHz at 112 mV/cm for 30 h; 86 MHz at 112 mV/cm for 30 h; 94 MHz at 112 mV/cm for 20 h.

The activity of the activated yeast cells towards decoquinatone was determined by measuring the amounts of decoquinatone the activated yeast cells can degrade. Two 100 liter samples each containing decoquinatone concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28 °C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of decoquinatone remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of decoquinatone in the samples with the activated yeast cells was reduced by more than 67.9%.

Yeast cell component that decomposes Trichlorophenol compounds

In yet another specific embodiment, a method for producing yeast cells that decompose trichlorophenol and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 220.000 to 250.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, and 250 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.100 were cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 74 MHz at 219 mV/cm for 30 h; 86 MHz at 219 mV/cm for 20 h; 96 MHz at 219 mV/cm for 30 h; 98 MHz at 219 mV/cm for 20 h.

The activity of the activated yeast cells towards trichlorophenol was determined by measuring the amounts of trichlorophenol the activated yeast cells can degrade. Two 100 liter samples each containing trichlorophenol concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated

yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of trichlorophonum remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of trichlorophonum in the samples with the activated yeast cells was reduced by more than 72.4%.

Yeast cell component that decomposes Dinitolmide

In a specific embodiment, method for producing yeast cells that decompose dinitolmide and related compounds is provided. Dinitolmide is 2-methyl-3,5-dinitrobenzamide and is also known as zoalene. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 220.000 to 250.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, and 250 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.189 are cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 202 mV/cm for 30 h; 82 MHz at 202 mV/cm for 30 h; 90 MHz at 202 mV/cm for 20 h; 96 MHz at 202 mV/cm for 20 h.

The activity of the activated yeast cells towards dinitolmide was determined by measuring the amounts of zoalene compounds the activated yeast cells can degrade. Two 100 liter samples each containing a dinitolmide concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of dinitolmide remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dinitolmide in the samples with the activated yeast cells was reduced by more than 72.4%.

Yeast cell component that removes ammonium compound (NH_4)

In a specific embodiment, a method for producing yeast cells that remove or reduce the level of ammonium compounds in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 660 to 680 MHz or 2160

to 2190 MHz, and preferably 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.614 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 662 MHz at 152 mv/cm for 18 h; 666 MHz at 152 mv/cm for 18 h; 672 MHz at 152 mv/cm for 18 h; 678 MHz at 152 mv/cm for 18 h; 662 MHz at 310 mv/cm for 25 h; 666 MHz at 310 mv/cm for 25 h; 672 MHz at 310 mv/cm for 35 h; 678 MHz at 310 mv/cm for 35 h.

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The activity of the activated yeast cells was determined by measuring the amounts of ammonium compounds removed by the activated yeast cells. The amount of ammonium compounds in the samples with the activated yeast cells was reduced significantly (>93.6%) compared to the sample containing unactivated yeast cells.

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Yeast cell component that removes nitrates and nitrites

In a specific embodiment, a method for producing yeast cells that remove or reduce the level of nitrates and nitrites in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 661.000 to 680.000 MHz including but not limited to 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, and 680 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.14 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 661 MHz at 126 mV/cm for 25 h; 665 MHz at 126 mV/cm for 25 h; 672 MHz at 126 mV/cm for 25 h; 676 MHz at 126 mV/cm for 25 h; 661 MHz at 196 mV/cm for 25 h; 665 MHz at 196 mV/cm for 25 h; 672 MHz at 196 mV/cm for 38 h; 676 MHz at 196 mV/cm for 38 h.

The activity of the activated yeast cells towards nitrates was determined by measuring the amounts of nitrates removed by the activated yeast cells. Two 100 liter samples each containing nitrates at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of nitrates remaining in the samples were determined and compared by performing HPLC

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on the samples. Comparing to samples with unactivated yeast cells, the amount of nitrates in the samples with the activated yeast cells was reduced by more than 69.7%

5 Yeast cell component that remove biologically available phosphorus

In a specific embodiment, a method for producing yeast cells that remove biologically available phosphorus, e.g., HPO_4^{2-} , H_2PO_4^- , etc., is provided. In a specific embodiment, a method for producing yeast cells that remove or reduce the level of nitrates and nitrites in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 80.000 to 440.000 MHz, preferably 86.000 to 120.000 MHz or 410.000 to 440.000 MHz including but not limited to 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, and 430 MHz. For example, Yeast cells of *Saccharomyces cerevisiae* strain AS2.620 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 98 MHz at 68 mv/cm for 24 h; 112 MHz at 68 mv/cm for 24 h; 108 MHz at 68 mv/cm for 24 h; 118 MHz at 68 mv/cm for 24 h; 98 MHz at 240 mv/cm for 24 h; 112 MHz at 240 mv/cm for 24 h; 108 MHz at 240 mv/cm for 42 h; 118 MHz at 240 mv/cm for 42 h.

The activity of the activated yeast cells towards available phosphorus was determined by measuring the amounts of available phosphorus the activated yeast cells can remove. Two 100 liter samples each containing available phosphorus concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of phosphorous remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of available phosphorus in the samples with the activated yeast cells was reduced by more than 65.8%.

Yeast cell component that decomposes Trichlorphon

In a specific embodiment, a method for producing yeast cells that decompose trichlorphon and related organophosphate pesticide compounds is provided. The

- frequencies of the EM field(s) used to activate the yeast cells are in the range of 1980.000 to 2020.000, and preferably 2000.000 to 2020.000 including but not limited to 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, and 2020 MHz. For example, yeast cells of *Saccharomyces*
- 5 *cerevisiae* strain AS2.440 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 2000 MHz at 125mv/cm for 10 h; 2004 MHz at 125 mv/cm for 10 h; 2009 MHz at 125 mv/cm for 24 h; 2018 MHz at 125 mv/cm for 24 h; 2000 MHz at 168 mv/cm for 10 h; 2004 MHz at 168mv/cm for 10 h; 2009 MHz at 168 mv/cm for 56 h; 2018 MHz at 168 mv/cm for 56 h.
- 10 The activity of the activated yeast cells towards trichlorphon was determined by measuring the amounts of trichlorphon the activated yeast cells can degrade. Two 100 liter samples each containing trichlorphon concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10⁷ cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C.
- 15 A control was included which did not contain any yeast cells. After 24 hours, the amount of trichlorphon remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of trichlorphon in the samples with the activated yeast cells was reduced by more than 10% in 48 hours.

20

Yeast cell component that decomposes Dichlorvos

- In a specific embodiment, a method for producing yeast cells that decompose dichlorvos (DDVP) and related organophosphate pesticide compounds is provided. The
- 25 frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043, 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces*
- 30 *cerevisiae* strain AS2.443 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 1993 MHz at 140 mV/cm for 24 h; 2023 MHz at 140 mV/cm for 24 h; 2083 MHz at 140 mV/cm for 24 h; 2103 MHz at 140 mV/cm for 24 h; 1993 MHz at 190 mV/cm for 24 h; 2023 MHz at 190 mV/cm for 24 h; 2083 MHz at 190 mV/cm for 56 h; 2103 MHz at 190 mV/cm for 56 h.

- The activity of the activated yeast cells towards dichlorvos was determined
- 35 by measuring the amounts of dichlorvos the activated yeast cells can degrade. Two 100 liter

samples each containing dichlorvos concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of
5 dichlorvos remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dichlorvos in the samples with the activated yeast cells was reduced by more than 67.5%.

Yeast cell component that decomposes Momocrotophos

10

In a specific embodiment, a method for producing yeast cells that decompose momocrotophos and related insecticides is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043,
15 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.93 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 2998 MHz at 165 mV/cm for 24 h; 2033 MHz at 165 mV/cm for 24 h; 2058 MHz at 165 mV/cm for 24 h; 2113 MHz at 165 mV/cm for 24 h;
20 2998 MHz at 202 mV/cm for 56 h; 2033 MHz at 202 mV/cm for 56 h; 2058 MHz at 202 mV/cm for 24 h; 2113 MHz at 202 mV/cm for 24 h.

The activity of the activated yeast cells towards momocrotophos was determined by measuring the amount of momocrotophos the activated yeast cells can degrade. Two 100 liter samples each containing momocrotophos concentration of 100mg/L
25 were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of monocrotophos remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated
30 yeast cells, the amount of momocrotophos in the samples with the activated yeast cells was reduced by more than 73.4%.

35

Yeast cell component that decomposes Dimethoate

In a specific embodiment, a method for producing yeast cells that decompose dimethoate and related insecticidal compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043, 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.379 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 1988 MHz at 195 mV/cm for 24 h; 2023 MHz at 195 mV/cm for 24 h; 2088 MHz at 195 mV/cm for 24 h; 2108 MHz at 195 mV/cm for 24 h; 1988 MHz at 277 mV/cm for 56 h; 2023 MHz at 277 mV/cm for 56 h; 2088 MHz at 277 mV/cm for 24 h; 2108 MHz at 277 mV/cm for 24 h.

The activity of the activated yeast cells towards dimethoate was determined by measuring the amount of dimethoate the activated yeast cells can degrade. Two 100 liter samples each containing dimethoate concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of dimethoate remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dimethoate in the samples with the activated yeast cells was reduced by more than 69.6%.

Yeast cell component that decomposes DDT

In a specific embodiment, a method for producing yeast cells that decompose DDT and related dilorinated organic insecticidal compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1420.000 to 1435.000 including but not limited to 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.415 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 1423 MHz at 75 mV/cm for 24 h; 1426 MHz at 75 mV/cm for 24 h; 1433 MHz at 75 mV/cm for 24 h; 1435 MHz at 75 mV/cm for 24 h; 1423 MHz at 146 mV/cm for 56 h; 1426 MHz at 146 mV/cm for 56 h; 1433 MHz at 146 mV/cm for 24 h; 1435 MHz at 146 mV/cm for 24 h.

The activity of the activated yeast cells towards DDT was determined by measuring the amount of DDT the activated yeast cells can degrade. Two 100 liter samples each containing DDT concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added
5 separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of DDT remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of DDT in the samples with the activated yeast cells was reduced by more than 78.5%.

10

Yeast cell component that decomposes Toxaphene

In a specific embodiment, a method for producing yeast cells that decompose toxaphene and related chlorinated organic insecticidal compounds is provided. The
15 frequencies of the EM field(s) used to activate the yeast cells are in the range of 1420.000 to 1435.000 including but not limited to 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.504 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order
20 stated: 1420 MHz at 120 mV/cm for 24 h; 1426 MHz at 120 mV/cm for 24 h; 1431 MHz at 120 mV/cm for 24 h; 1434 MHz at 120 mV/cm for 24 h.

The activity of the activated yeast cells towards toxaphene was determined by measuring the amount of toxaphene the activated yeast cells can degrade. Two 100 liter samples each containing toxaphene concentration of 100mg/L were prepared. Then, 0.1 ml
25 of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of toxaphene remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of
30 toxaphene in the samples with the activated yeast cells was reduced by more than 70.8%.

5.3. ODOR-REDUCING YEAST CELL COMPONENTS

The present invention also provides yeast cells that are capable of reducing the odor of solid waste, e.g., manures, sludge, and/or garbage. Without being bound by any
35 theory, the inventor believes that the yeast cells of the invention are capable of reducing the

odor of solid waste by modifying, assimilating, or decomposing known and unknown compounds in the solid waste that are malodorous. However, it is not necessary to demonstrate that such compounds have been decomposed. It is sufficient so long as the odor is reduced as determined subjectively by a panel of subjects, after the yeast cells of the
5 invention have been used.

According to the present invention, yeast cells that are capable of reducing the odor of solid waste are prepared by culturing the cells in the presence of an electromagnetic field in an appropriate culture medium. The frequency of the electromagnetic field for activating or enhancing this ability in yeasts can generally be found
10 in the range of 2160 to 2380 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their ability to reduce the odor of solid waste by methods well known in the art.

The method of the invention for making the odor-reducing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by
15 the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies
20 between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate,
25 and like ions. Non-limiting examples of nutrient inorganic salts are $(\text{NH}_4)_2\text{HPO}_4$, CaCO_3 , MgSO_4 , NaCl , and CaSO_4 .

It should be noted that the composition of the media provided in Table 38 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale
30 of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2 - 10^5 cell/ml, preferably 3×10^2 - 10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series
35 of EM fields are applied, the yeast culture can remain in the same container and use the

same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 2160 to 2380 MHz, and preferably in the ranges of 2160.000 to 2250.000 MHz or 2280.000 to 2380.000 MHz. The field strength of the EM field(s) is in the range of 25 to 300 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 80-320 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of 25-200 mV/cm. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the range of 250-300mV/cm. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m³, preferably 0.4 mol/m³. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0-4°C. The recovered yeast cells may also be dried and stored in powder form.

Any methods known in the art can be used to test the cultured yeast cells for their ability to reduce the odor of organic materials. The amount of malodorous chemicals such as hydrogen sulfide, ammonia, indole, p-cresol, skatol, and organic acids present in a test sample of organic material can be determined by any methods known in the art,

including but not limited to gas phase chromatography, olfactometry, mass spectrometry, or the use of an odor panel.

For example, to determine the activity of the activated yeast cells towards an malodorous compound, mass spectrometry (e.g., VG micromass) can be used to measure the amounts of the malodorous compound in a test sample at various time point and under different incubation conditions. For example, a known amount of a malodorus compound (up to 100 mg per liter) is added to 10 liter of an aqueous extract of manure. Then, 0.1 ml of activated and unactivated yeasts (at least 10^7 cells/ml) are added to the 10 liter samples containing the compound, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of the malodorous compounds remaining in the extracts are determined and compared.

Yeast cell component that reduce odor caused by sulfur containing compounds

In one embodiment of the invention, a method for producing yeast cells that remove hydrogen sulfide and other related sulfur-containing or sulfhydryl (SH-) containing molecules is provided. Yeast cells that remove hydrogen sulfide and other related sulfur-containing or sulfhydryl (SH-) containing molecules can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.559 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2165 MHz at 240 mV/cm for 20 hr; 2175 MHz at 240 mV/cm for 20-60 hr; 2200 MHz at 240 mV/cm for 20 hr; 2235 MHz at 240 mV/cm for 20 hr.

The activity of the activated yeast cells towards sulfur-containing or sulfhydryl (SH-) compounds was determined by measuring the change in amount of hydrogen sulfides in the presence of the activated yeast cells. Two 100 liter samples each containing hydrogen sulfide concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of hydrogen sulfide remaining in the samples were determined and compared. Comparing to

samples with unactivated yeast cells, the amount of hydrogen sulfide in the samples with the activated yeast cells was reduced by more than 59.8%.

Yeast cell component that reduce odor caused by NH-containing containing compounds

5

In another embodiment of the invention, a method for producing yeast cells that remove ammonia and related NH-containing compounds is provided. Yeast cells that remove ammonia and related NH-containing compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.423 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2160 MHz at 250 mV/cm for 20 hr; 2175 MHz at 250 mV/cm for 20 hr; 2210 MHz at 250 mV/cm for 20 hr; 2245 MHz at 250 mV/cm for 10 hr.

The activity of the activated yeast cells towards ammonia acid NH-containing compounds was determined by measuring the change in the amount of ammonia in the presence of the activated yeast cells. Two 100 liter samples each containing NH-containing compounds at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10⁷ cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of ammonia remaining in the samples were determined and compared. Comparing to samples with unactivated yeast cells, the amount of ammonia in the samples with the activated yeast cells was reduced by more than 69.6%.

Yeast cell component that reduce odor caused by indole and other related compounds

30

In the invention, a method for producing yeast cells that decompose indole and other related compounds, such as skatol is provided. Yeast cells that decompose indole and other related compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.612 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2165 MHz at 240 mV/cm for 40 hr; 2180 MHz at 240 mV/cm for 20 hr; 2200 MHz at 240 mV/cm for 40 hr; 2220 MHz at 240 mV/cm for 20
5 hr.

The activity of the activated yeast cells towards indole and other related compounds was determined by measuring the amount of indole removed by the activated yeast cells. Two 100 liter samples each containing indole related compounds at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml
10 of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of indole remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of indole in the samples with the activated yeast
15 cells was reduced by more than 71.3%.

Yeast cell component that reduce odor caused by organic acids

In yet another embodiment of the invention, a method for producing yeast
20 cells that remove odorous organic acids, e.g., formic acid, acetic acid, propanoic acid, butyric acid, and other volatile fatty acids, is provided. Yeast cells that can reduce the odor of organic acids can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240,
25 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.53 are cultured at about 25-30°C in a culture medium as described in Table 42 in the presence of a series of 4 EM fields in the order stated: 2315 MHz at 290 mV/cm for 30 hr; 2335 MHz at 290 mV/cm for 10 hr; 2355 MHz at 290 mV/cm for 20 hr; 2375 MHz at 290 mV/cm for 10
30 hr.

The activity of the activated yeast cells towards organic acids was determined by measuring the change in the amounts of acetic acid in the presence of the activated yeast cells. Two 100 liter samples each containing organic acids concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated
35 yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and

incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of acetic acid remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of acetic acid in the samples with the activated yeast
5 cells was reduced by more than 89.4%.

Yeast cell component that reduce odor caused by aliphatic substituted amine

In yet another embodiment of the invention, a method for producing yeast
10 cells that remove or degrade aliphatic substituted amine, such as methylamine, dimethylamine, or trimethylamine thereby reducing the odor caused by such compounds, is provided. Yeast cells that remove or degrade such amines can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205,
15 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.541 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2160 MHz at 250 mV/cm for 20 hr; 2190 MHz at 250 mV/cm for 10 hr; 2210 MHz at 250 mV/cm for 40 hr; 2250 MHz at 250 mV/cm for 40
20 hr.

The activity of the activated yeast cells towards methyl-substituted amine was determined by measuring the amount of such amine in the presence of the activated yeast cells. Two 100 liter samples each containing methyl-substituted amine at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml
25 of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of methyl-substituted amines remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of methyl-substituted amines in the
30 samples with the activated yeast cells was reduced by more than 82.2%.

Yeast cell component that reduce odor caused by p-cresol and related compounds

In yet another embodiment of the invention, a method for producing yeast
35 cells that remove or degrade p-cresol and related compounds is provided. Yeast cells that

remove or degrade p-cresol and other related compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

5 For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.163 were cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2300 MHz at 98 mV/cm for 20 hr; 2370 MHz at 98 mV/cm for 15 hr; 2300 MHz at 250 mV/cm for 20 hr; 2370 MHz at 250 mV/cm for 30 hr.

 The activity of the activated yeast cells towards p-cresol and related
10 compounds was determined by measuring the change in the amounts of p-cresol and related compounds in the presence of the activated yeast cells. Two 100 liter samples each containing p-cresol at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was
15 included which did not contain any yeast cells. After 24 hours, the amounts of p-cresol remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of p-cresol in the samples with the activated yeast cells was reduced by more than 92.5%.

20 5.4. PATHOGEN-SUPPRESSING YEAST CELL COMPONENTS

 The present invention also provides yeast cells that are capable of suppressing the proliferation of pathogenic microorganisms that are present in solid waste. Typically, due to an abundance of nutrients present in solid waste for such pathogenic microorganisms, the numbers of pathogens increase rapidly over a period of time.

25 However, in the presence of the pathogen-suppressing yeasts of the invention, the numbers of pathogens in the treated solid waste remains unchanged, or decreases over time. Without being bound by any theory or mechanism, the inventor believes that the presence of the pathogen-suppressing yeasts in the solid waste creates an environment that is unfavorable for the growth of pathogenic microorganisms.

30 According to the invention, the ability of yeasts to affect/control the numbers of pathogens is activated or enhanced by culturing the yeasts in the presence of an electromagnetic field. The resulting pathogen-suppressing yeast cells are used as a component in the solid waste treatment compositions of the invention.

 The frequency of the electromagnetic field for activating or enhancing the
35 ability of yeasts to control the numbers of pathogenic microorganisms can generally be

found in the range of 30 MHz to 50 MHz. After sufficient time is given for the yeast cells to grow, the cells can be tested for their ability to affect/control the number of pathogens by methods well known in the art.

The method of the invention for making pathogen-suppressing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(\text{NH}_4)_2\text{HPO}_4$, CaCO_3 , MgSO_4 , NaCl , and CaSO_4 .

Table 4: Composition for a culture medium for Pathogen-Suppressing yeasts

Medium Composition	Quantity
Soluble Starch	8.0g
Sucrose	5g
NaCl	0.2g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	0.5g
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.2g
Peptone	1.5g
K_2HPO_4	0.5g
Autoclaved water	400ml
Pathogen extract	600ml

The pathogen extract for the culture medium is prepared by incubating 500g of pathogen-containing waste in about 600ml of warm water (at 35°C to 40°C) for 24 hours

at 30-37°C, and filtering the fluid to remove particulate matters. It should be noted that the composition of the media provided in Table 4 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of
5 media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2 - 10^5 cell/ml, preferably 3×10^2 - 10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series
10 of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 30.000 to 50.000, 500.000 to 650.000, and 1000.000
15 to 1150.000 MHz. The field strength of the EM field(s) is in the range of 20 to 200 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM
20 field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of
25 culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 144 - 272 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an
30 output amplitude of the EM wave in the range of 20-180 mV/cm. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the range of 200-350 mV/cm.

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At the end of the culturing process, the pathogen-suppressing yeast cells may be recovered from the culture by various methods known in the art, and stored at about 0°C to 4°C. The pathogen-suppressing yeast cells may also be dried and stored in powder form.

The ability of the pathogen-suppressing yeasts to control the numbers of pathogens can be determined by any methods known in the art for enumerating microorganisms, such as optical density, plating out dilutions on solid media for counting, or counting individual cells under a microscope. Stains may be applied to distinguish or identify different strains or species of microorganisms present in a sample, or to determine their viability. When a range of pathogenic microorganisms are expected to be affected by the pathogen-suppressing yeasts, the numbers of more than one representative species of pathogenic microorganisms can be monitored to assess the performance of the pathogen-suppressing yeasts.

For example, samples of solid waste containing a known concentration of pathogenic microorganisms are cultured under the same conditions for a same period of time in the presence of different concentrations of pathogen-suppressing yeasts, and as negative control, the same strain of yeasts that have not been treated according to the culturing methods of the invention. A sample without any added yeast may also be included to determine the growth of pathogens under normal circumstances. The numbers of pathogens before and after the culture period are determined and compared.

A one liter culture containing at least 10^{10} cells of a pathogenic microorganism per ml is prepared. One ml of activated yeast cells (containing 2 to 5×10^7 yeasts per ml) is added to the one liter culture of pathogenic microorganism and incubated at 30°C for 24 hours. A control is included which contained unactivated yeast cells. The numbers of microorganisms in the respective culture is then determined and compared. The following are several examples of which a particular species of pathogenic bacteria was studied.

Yeast cell component that suppresses *Staphylococcus aureus*

In a specific embodiment of the invention embodiment, a method for producing yeast cells that suppress the growth of *Staphylococcus aureus* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.595 were cultured at about 25-30°C in a culture medium as described

in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for 12h; 43 MHz at 26 mV/cm for 12 h; 47 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 43 MHz at 150 mV/cm for 24 h; 47 MHz at 150 mV/cm for 24 h.

5 The activity of the activated yeast cells towards *Staphylococcus aureus* was determined by measuring the growth of *Staphylococcus aureus* in the presence of the activated yeast cells. *Staphylococcus aureus* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added
10 separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Staphylococcus aureus* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 2.6%.

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Yeast cell component that suppresses *Diplococcus pneumoniae*

In a specific embodiment, a method for producing yeast cells that suppress the growth of *Diplococcus pneumoniae* is provided. The frequencies of the EM field(s) used
20 to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain IFFI1021 were cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for
25 12h; 42 MHz at 26 mV/cm for 12 h; 49 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 42 MHz at 150 mV/cm for 24 h; 49 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *Diplococcus pneumoniae* was determined by measuring the growth of *Diplococcus pneumoniae* in the presence of the
30 activated yeast cells. *Diplococcus pneumoniae* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the
35 cell count of *Diplococcus pneumoniae* in the samples were determined by conventional

bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 3 %.

Yeast cell component that suppresses *Bacillus anthracis*

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In a specific embodiment, a method for producing yeast cells that suppress the growth of *Bacillus anthracis* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 20.000 to 45.000 MHz, including but not limited to 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 10 42, 43, 44, and 45 MHz. For example, yeast cells of *Bacillus anthracis* strain AS2.390 are cultured at about 25-30 °C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 24 MHz at 100 mv/cm for 24 h, 37 MHz at 100 mv/cm for 24 h, 40 MHz at 100 mv/cm for 24 h, 45 MHz at 100 mv/cm for 24 h, 24 MHz at 190 mv/cm for 24 h, 37 MHz at 190 mv/cm for 24 h, 40 MHz at 190 mv/cm for 24 h, 45 15 MHz at 190 mv/cm for 24 h.

The activity of the activated yeast cells towards *Bacillus anthracis* was determined by measuring the growth of *Bacillus anthracis* in the presence of the activated yeast cells. *Bacillus anthracis* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of 20 unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Bacillus anthracis* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples 25 with the activated yeast cells was reduced by 2.6 %.

Yeast cell component that suppresses *Mycobacterium tuberculosis*

In a specific embodiment, a method for producing yeast cells that suppress 30 *Mycobacterium tuberculosis* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.431 are cultured at about 25-30 ° in a culture medium as described in Table 4 in the presence of a series of 8 EM fields 35 in the order stated: 33 MHz at 26 mv/cm for 12 h; 36 MHz at 26 mv/cm for 12 h; 45 MHz

at 26 mv/cm for 12 h; 47 MHz at 26 mv/cm for 12 h; 33 MHz at 150 mv/cm for 24 h; 36 MHz at 150 mv/cm for 24 h; 45 MHz at 150 mv/cm for 24 h; 47 MHz at 150 mv/cm for 24 h.

The activity of the activated yeast cells towards *Mycobacterium tuberculosis* was determined by measuring the growth of *Mycobacterium tuberculosis* in the presence of the activated yeast cells. *Mycobacterium tuberculosis* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Mycobacterium tuberculosis* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 2.9 %.

15 Yeast cell component that suppresses *E. coli*

In a specific embodiment, a method for producing yeast cells that suppress the growth of *E. coli* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.561 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 34 MHz at 26 mV/cm for 12 h; 38 MHz at 26 mV/cm for 12 h; 49 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 34 43 MHz at 150 mV/cm for 24 h; 38 MHz at 150 mV/cm for 24 h; 49 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *E. coli* was determined by measuring the growth of *E. coli* in the presence of the activated yeast cells. *E. coli* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *E. coli* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with

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unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 48%.

Yeast cell component that suppresses *Salmonella*

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In a specific embodiment, a method for producing yeast cells that suppress the growth of *Salmonella* species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50
10 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.178 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 33 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for 12 h; 38 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 33 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 38 MHz at 150
15 mV/cm for 24 h.

The activity of the activated yeast cells towards *Salmonella* species was determined by measuring the growth of *Salmonella* in the presence of the activated yeast cells. *Salmonella* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts
20 cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Salmonella* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was
25 reduced by 62 %.

Yeast cell component that suppresses *Vibrio* species

In a specific embodiment, a method for producing yeast cells that suppress
30 *Vibrio* species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 500.000 to 550.000 MHz, including but not limited to 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, and 540 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.377 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a
35 series of 8 EM fields in the order stated: 521 MHz at 150 mv/cm for 24 h, 527 MHz at 150

mv/cm for 24 h, 531 MHz at 150 mv/cm for 24 h, 538 MHz at 150 mv/cm for 24 h, 521 MHz at 276 mv/cm for 24 h, 527 MHz at 276 mv/cm for 24 h, 531 MHz at 276 mv/cm for 24 h, 538 MHz at 276 mv/cm for 24 h.

The activity of the activated yeast cells towards *Vibrio* species was
 5 determined by measuring the growth of *Vibrio* species in the presence of the activated yeast cells. *Vibrio* species contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was
 10 included which did not contain any yeast cells. After 24 hours, the cell count of *Vibrio* species in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 5.6 %.

15 Yeast cell component that suppresses Shigella species

In a specific embodiment, a method for producing yeast cells that suppress *Shigella* species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 600.000 to 650.000 MHz, including but not limited to 630, 631,
 20 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, and 650 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.395 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 630 MHz at 180 mv/cm for 24 h, 636 MHz at 180 mv/cm for 24 h, 641 MHz at 180 mv/cm for 24 h, 649 MHz at 180 mv/cm for 24 h, 630
 25 MHz at 314 mv/cm for 24 h, 636 MHz at 314 mv/cm for 24 h, 641 MHz at 314 mv/cm for 24 h, 649 MHz at 314 mv/cm for 24 h.

The activity of the activated yeast cells towards *Shigella* species was determined by measuring the growth of *Shigella* species in the presence of the activated yeast cells. *Shigella* species contained in an extract from solid waste was grown in a culture
 30 until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Shigella* species in the samples were determined by conventional bacteria cell counting
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method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 4.6 %.

Yeast cell component that suppresses *Clostridium botulinum*

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In a specific embodiment, a method for producing yeast cells that suppress *Clostridium botulinum* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1000.000 to 1050.000 MHz, including but not limited to 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023,
10 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1034, and 1035 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.432 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 1012 MHz at 180 mv/cm for 24 h, 1018 MHz at 180 mv/cm for 24 h, 1024 MHz at 180 mv/cm for 24 h, 1033 MHz at 180 mv/cm for 24 h, 1012 MHz at 323
15 mv/cm for 24 h, 1018 MHz at 323 mv/cm for 24 h, 1024 MHz at 323 mv/cm for 24 h, 1033 MHz at 323 mv/cm for 24 h.

The activity of the activated yeast cells towards *Clostridium botulinum* was determined by measuring the growth of *Clostridium botulinum* in the presence of the activated yeast cells. *Clostridium botulinum* contained in an extract from solid waste was
20 grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Clostridium botulinum* in the samples were determined by conventional
25 bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 5.1 %.

Yeast cell component that suppresses *Bacillus aerogenes capsulatus*

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In a specific embodiment, a method for producing yeast cells that suppress *Bacillus aerogenes capsulatus*. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1100.000 to 1150.000 MHz, including but not limited to 1100, 1101, 1102, 1103, 1104, 1105, 1106, 1107, 1108, 1109, 1110, 1111, 1112, 1113, 1114, 1115, 1116, 1117, 1118, 1119, and 1120 MHz. For example, yeast cells of *Saccharomyces*
35 *cerevisiae* strain AS2.432 are cultured at about 25-30°C in a culture medium as described in

Table 4 in the presence of a series of 8 EM fields in the order stated: 1102 MHz at 180 mv/cm for 24 h, 1106 MHz at 180 mv/cm for 24 h, 1113 MHz at 180 mv/cm for 24 h, 1117 MHz at 180 mv/cm for 24 h, 1102 MHz at 301 mv/cm for 24 h, 1106 MHz at 301 mv/cm for 24 h, 1113 MHz at 301 mv/cm for 24 h, 1117 MHz at 301 mv/cm for 24 h.

- 5 The activity of the activated yeast cells towards *Bacillus aerogenes capsulatus* was determined by measuring the growth of *Bacillus aerogenes capsulatus* in the presence of the activated yeast cells. *Bacillus aerogenes capsulatus* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Bacillus aerogenes capsulatus* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 6.2 %.

5.5 ADAPTATION

- In another embodiment of the invention, activated yeast cells prepared according to any one of Sections 5.1-5.10 can be further cultured as a mixture in the presence of a sample of the solid waste which is to be treated. This optional process which improves the performance of the solid waste treatment compositions is described by way of an example as follows.

- An extract of the solid waste to be treated, such as manure or sludge, is prepared by mixing and soaking about 1000 g of poultry manure in 1000 to 3000 ml of water for about 48 hours. The extract is then mixed with about 1000 g of dried manure (dry weight, i.e., less than 10% moisture) to form a suspension to which the yeast cells are added. At least 1 ml of yeasts which contains more than 5×10^7 cell/ml is added to the suspension. Depending on the number of strains of activated yeast cells used, up to about 50 ml of yeast cells can be added. If only a few strains are used, 5 to 10ml of yeast cells per strain can be added. The process can be scaled up or down according to needs. The mixture of yeast and solid waste is cultured for about 120-280 hours in the presence of a series of electromagnetic fields. Each electromagnetic field has a frequency that, depending on the strains of yeast included, corresponds to one of the frequencies described in Sections 5.1-5.4. If many different strains of yeasts are used, a combination of the following five frequency bands can be used : 20-50 MHz, 60-150 MHz, 400-700 MHz, 1400-1600 MHz,

2000-2500 MHz; each for about 24 to 56 hours. Generally, the yeast cells are subjected to an EM field strength in the range from 20mV/cm to 350mV/cm in this process.

The culture is incubated at temperatures that cycle between about 5°C to about 37°C. For example, in a typical cycle, the temperature of the culture may start at about 37°C and be kept at this temperature for about 1-2 hours, then adjusted to 26-30°C and kept at this temperature for about 2-4 hours, and then brought down to 5-10°C and kept at this temperature for about 1-2 hours, and then the temperature may be raised again to about 37°C for another cycle. The cycles are repeated until the process is completed. After the last temperature cycle is completed, the temperature of the culture is lowered to 3-4°C and kept at this temperature for about 5-6 hours. After the process, the yeast cells may be isolated and recovered from the medium by conventional methods, such as filtration. The adapted yeast cells can be stored under 4°C. An exemplary set-up of the culture process is depicted in Figure 2.

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5.6 MANUFACTURE OF THE BIOLOGICAL COMPOSITIONS

The biological composition of the present invention can be produced by culturing yeast cells under appropriate conditions according to Section 5.1 to 5.4, and mixing the desired amounts of cultures of yeast cells. Since the biological composition is not immediately used to treat solid waste, the yeasts of the biological composition can be dried in a two-stage drying process. During the first drying stage, the yeast cells are dried in a first dryer at a temperature not exceeding 65°C for a period of time not exceeding 10 minutes so that yeast cells quickly become dormant. The yeast cells are then sent to a second dryer and dried at a temperature not exceeding 70°C for a period of time not exceeding 30 minutes to further remove water. After the two stages, the water content should be lower than 5%. It is preferred that the temperatures and drying times be adhered to in both drying stages so that yeast cells do not lose their vitality and functions. The dried yeast cells are then cooled to room temperature. The dried yeast cells may also be screened in a separator so that particles of a preferred size are selected. The dried cells can then be sent to a bulk bag filler for packing.

The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of

the invention. Indeed various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

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WHAT IS CLAIMED IS:

1. A method for treatment of solid waste comprising antibiotics, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the antibiotics, wherein said plurality of yeast cells comprises at least one of the following:

- (a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 100 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of penicillin, chlortetracycline, oxytetracycline, doxycycline, tetracycline, streptomycin, kanamycin, erythromycin, spiramycin and bacitracin;
- (b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 410 to 470 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of colistin, chloramphenicol, and cephalothin; or
- (c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 550 to 620 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of neomycin and novobiocin.

2. A method for treatment of solid waste comprising undesirable chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following::

- (a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 52 to 98 MHz and a field strength of 8 to 300 mV/cm, which can degrade toluene, ethylbenzene, or trichlorophenol;
- (b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 30 to 50 MHz or 70 to 98 MHz and a field strength of 8 to 250 mV/cm; and which can degrade dimethylbenzene compounds;

- (c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 133 to 151 MHz and a field strength of 8 to 250 mV/cm, and which can degrade benzaldehyde;
- 5 (d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 145 to 162 MHz and a field strength of 8 to 250 mV/cm, and which can degrade propylaldehyde;
- 10 (e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 100 MHz and a field strength of 8 to 250 mV/cm, and which can degrade nenanthaldehyde; and
- 15 (f) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 163 to 183 MHz and a field strength of 8 to 250 mV/cm, and which can degrade dichlorobenzene.

3. A method for treatment of solid waste comprising undesirable chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, 20 allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

- (a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 175 to 191 MHz and a field strength of 8 to 250 mV/cm, 25 and which can degrade acetophenone;
- (b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 183 to 205 MHz and a field strength of 8 to 250 mV/cm, and which can degrade arsanilic acid;
- 30 (c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 114 to 128 MHz and a field strength of 8 to 250 mV/cm, and which can degrade is roxarsone;
- 35 (d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of

70 to 98 MHz or 200 to 220 MHz and a field strength of 8 to 250 mV/cm and which can degrade furazolidonum;

(e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 213 to 229 MHz and a field strength of 8 to 250 mV/cm and which can degrade decoquinat; and

(f) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 220 to 250 MHz and a field strength of 8 to 250 mV/cm, and which can degrade trichlorophonum or dinitomide.

4. A method for treatment of solid waste comprising undesirable chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 660 to 680 MHz or 2160 to 2190 MHz and a field strength of 25 to 300 mV/cm, and which can reduce the amount of ammonium compounds;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 661 to 680 MHz and a field strength of 8 to 250 mV/cm, and which can reduce the amount of nitrites or nitrates; or

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 86 to 120 MHz or 410 to 430 MHz and a field strength of 8 to 250 mV/cm, and which can reduce the amount of phosphates.

5. A method for treatment of solid waste comprising undesirable chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1980 to 2118 MHz and a field strength of 25 to 300mV/cm, and which can

degrade undesirable chemical selected from the group consisting of trichlorophon, dichlorvos, momocrotophos and dimethoate;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1420 to 1435 MHz and a field strength of 25 to 300mV/cm, and which can degrade DDT or toxaphene.

6. A method for reducing the odor of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of odorous molecules in the solid waste, wherein said yeast are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 2160 to 2380 MHz and a field strength of 25 to 300 mV/cm, and said odorous molecules are selected from the group consisting of hydrogen sulfide, ammonia, indole, skatol, acetic acid, methylamine, and p-cresol.

7. A method for treatment of solid waste comprising pathogenic bacteria, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to suppress the growth of pathogenic bacteria in the solid waste, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 20 to 50 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of *Staphylococcus aureus*, *Diplococcus pneumoniae*, *Bacillus anthracis*, *Mycobacterium tuberculosis*, *Salmonella* species, or *E. coli*;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 500 to 550 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of *Vibrio* species;

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 600 to 650 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of *Shigella* species;

(d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of

1000 to 1050 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of *Clostridium botulinum*; and

(e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1100 to 1150 MHz and a field strength of 20 to 50 mV/cm, and which can suppress the growth of *Bacillus aerogenes capsulatus*.

8. A method for treatment of solid waste comprising adding a biological composition to the solid waste, said biological composition comprising at least one of the following yeast cell components:

(a) a first yeast cell component comprising a plurality of yeast cells that degrade antibiotics in solid waste, said first yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range selected from the group consisting of 70 to 100 MHz, 410 to 470 MHz, and 550 to 620 MHz and a field strength of 8 to 250 mV/cm;

(b) a second yeast cell component comprising a plurality of yeast cells that degrade undesirable chemicals in solid waste, said second yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range selected from the group consisting of 30 to 100 MHz, 70 to 280 MHz, 410 to 430 MHz, 660 to 680 MHz and 1980 to 2210 MHz and a field strength of 8 to 250 mV/cm;

(c) a third yeast cell component comprising a plurality of yeast cells that reduce the amount of odorous molecules in solid waste, said third yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 2160 to 2380 MHz and a field strength of 25 to 300 mV/cm;

(d) a fourth yeast cell component comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in the solid waste, said fourth yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range selected from the group consisting of 30 to 50 MHz, 500 to 550 MHz, 600 to 650 MHz, 1000 to 1050 MHz, and 1100 to 1150 MHz and a field strength of 20 to 350 mV/cm; and

allowing the yeast cells in the yeast cell component(s) to reduce the amount of antibiotics, undesirable chemicals, odorous compounds and pathogenic bacteria in the solid waste.

9. The method of claim 8, wherein the biological composition comprises the yeast cell components of (a), (b), (c) and (d).

10. The method of claim 8 wherein said yeast cells are cells of a species
5 of yeast selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Saccharomyces delbrueckii*, *Saccharomyces exiguus*, *Saccharomyces fermentati*, *Saccharomyces logos*, *Saccharomyces mellis*, *Saccharomyces microellipsoides*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Saccharomyces rouxii*, *Saccharomyces sake*, *Saccharomyces uvarum* Beijer, *Saccharomyces willianus*, *Saccharomyces ludwigii*,
10 *Saccharomyces sinenses*, and *Saccharomyces carlsbergensis*.

11. The method of claim 8 wherein said yeast cells are *Saccharomyces cerevisiae* cells.

12. The method of claim 11 wherein said biological composition
15 comprises dried yeast cells, and about 300 to 600g of the biological composition is added per cubic meter of solid waste.

13. The method of claim 11 wherein prior to adding said dried yeast cells
20 to said solid waste, said dried yeast cells are mixed with water in the ratio of about 1000 g yeast cells to about 30 liters, and incubated for about 12 to 24 hours.

14. A composition comprising a plurality of yeast cells that degrade
antibiotics in solid waste, wherein said plurality of yeast cells is prepared by a method
25 comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range selected from the group consisting of 70 to 100 MHz, 410 to 470 MHz, and 550 to 620 MHz and (ii) a field strength of 8 to 250 mV/cm.

15. A composition comprising a plurality of yeast cells that degrade
undesirable chemicals in solid waste, wherein said plurality of yeast cells is prepared by a
method comprising culturing yeast cells in an electromagnetic field or a series of
electromagnetic fields having (i) one or more frequencies in the range selected from the
group consisting of 30 to 100 MHz, 70 to 280 MHz, 410 to 430 MHz, 660 to 680 MHz and
30 1980 to 2210 MHz and (ii) a field strength of 8 to 250 mV/cm.
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16. A composition comprising a plurality of yeast cells that reduce the odor of solid waste, wherein said plurality of yeast cells is prepared by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having
5 (i) one or more frequencies in the range of 2160 to 2380 MHz and (ii) a field strength of 25 to 300 mV/cm;

17. A composition comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in solid waste, wherein said plurality of yeast cells is prepared
10 by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range selected from the group consisting of 30 to 50 MHz, 500 to 550 MHz, 600 to 650 MHz, 1000 to 1050 MHz, and 1100 to 1150 MHz and (ii) a field strength of 20 to 350 mV/cm.

15 18. The composition of claim 14, 15, 16, or 17, wherein said yeast cells are cells of a species of yeast selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Saccharomyces delbrueckii*, *Saccharomyces exiguus*, *Saccharomyces fermentati*, *Saccharomyces logos*, *Saccharomyces mellis*, *Saccharomyces microellipsoides*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Saccharomyces rouxii*,
20 *Saccharomyces sake*, *Saccharomyces uvarum* Beijer, *Saccharomyces willianus*, *Saccharomyces ludwigii*, *Saccharomyces sinenses*, and *Saccharomyces carlsbergensis*.

19. The composition of claim 14, 15, 16, or 17, wherein said yeast cells are dried yeast cells.
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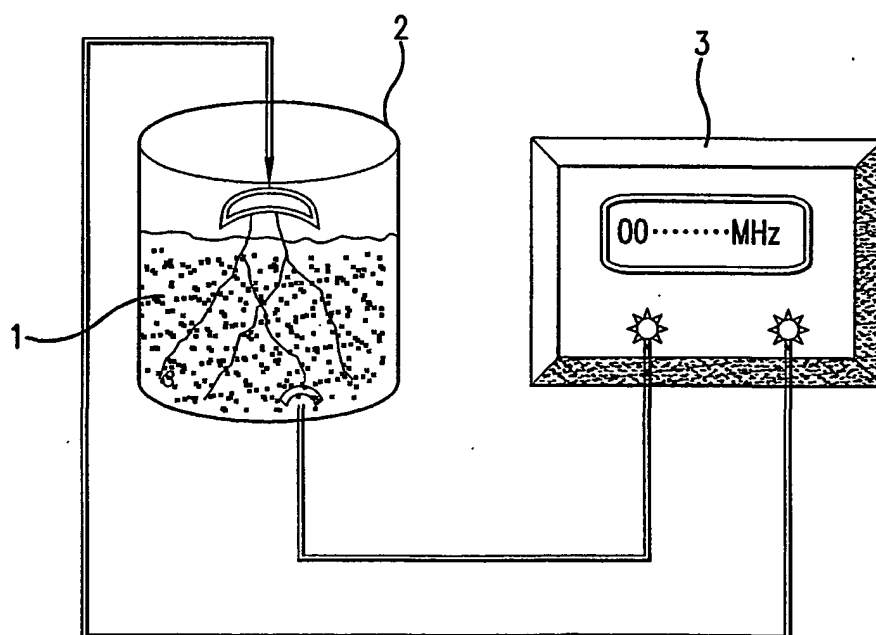
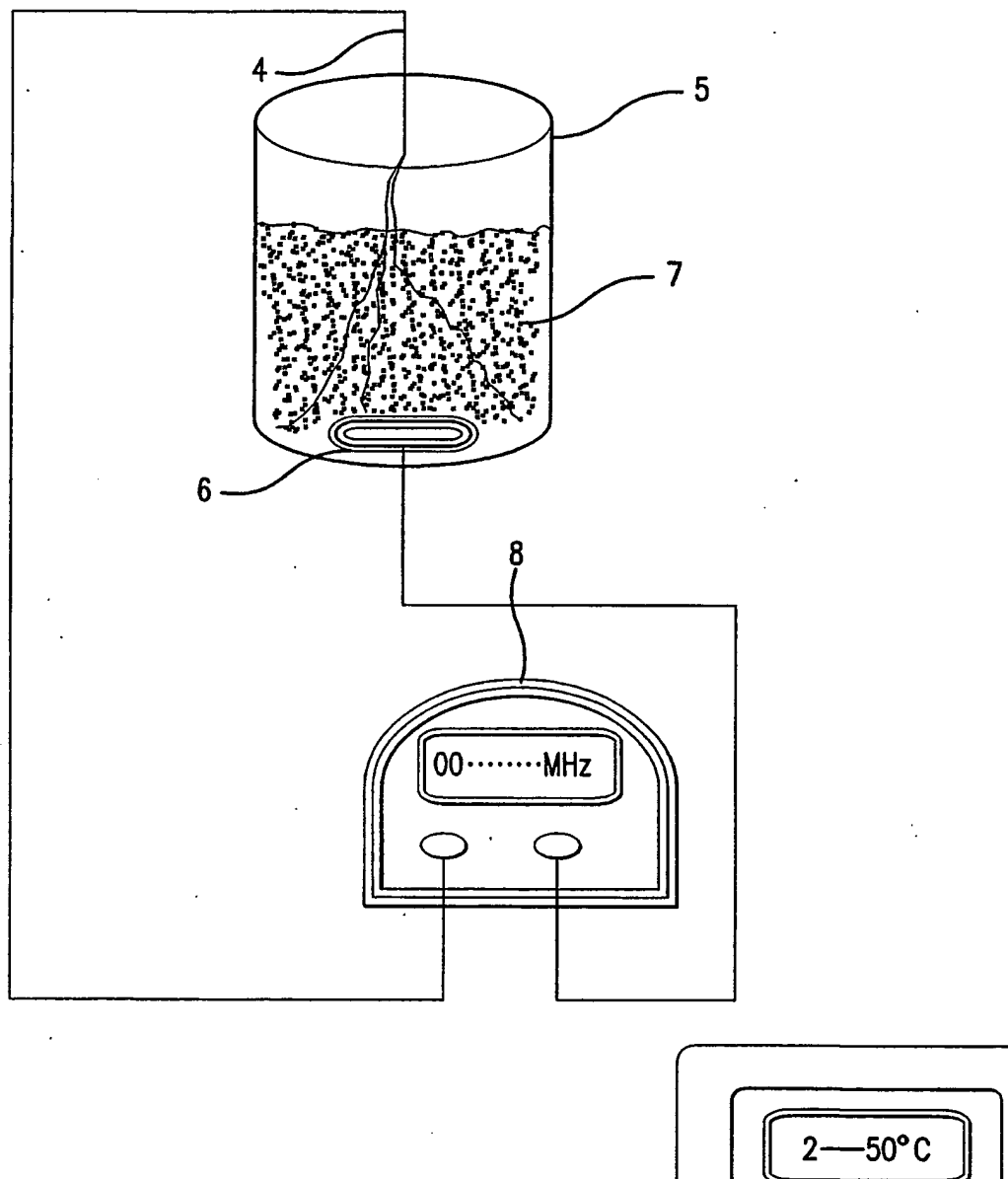


FIG.1

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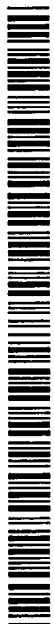
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(54) Title: BIOLOGICAL COMPOSITIONS FOR SOLID WASTE TREATMENT

(57) Abstract: The present invention relates to biological compositions useful for the treatment of solid waste. The biological compositions of the invention comprises a plurality of yeast cells which is capable of suppression of growth of pathogenic microor-
ganisms, breakdown of undesirable chemicals, such as antibiotics, insecticides and waste chemicals, and reducing the odor of organic
waste matters. The yeast cells of the invention are produced by culturing the plurality of yeast cells under activation conditions in the
presence of a series of electromagnetic fields. The invention also relates to methods for manufacturing the treatment composition.



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BIOLOGICAL COMPOSITIONS FOR SOLID WASTE TREATMENT

1. FIELD OF THE INVENTION

5 The invention relates to biological compositions that comprise yeasts for the treatment of solid waste. The yeasts in the compositions of the invention have been stimulated to perform a variety of functions including degradation of chemicals, reduction of odor and suppression of microorganisms. The invention also relates to methods for manufacturing the biological compositions, and methods for using the biological
10 compositions to treat waste.

2. BACKGROUND OF THE INVENTION

 Large amounts of solid waste are generated daily by industrial and agricultural activities, and by municipalities. If the waste is not treated properly, it can
15 cause severe and long-lasting damage to the environment. In 1995-1996, 208 million tons of municipal solid waste were generated in the United States. Of the municipal solid waste generated, 56 million tons (27 percent) were recovered by recycling or composting, 33.5 million tons (16 percent) were combusted at high temperatures, and 118.5 million tons (57 percent) were landfilled.

20 Municipal waste may be treated instead of landfilled. One type of municipal waste treatment involves high temperature burning of the waste in an incinerator. This combustion of municipal waste significantly reduces its volume. The ash from municipal waste combustion must be properly managed to prevent the environmental damage from any potential hazardous constituents. Also, emissions from the incinerator's smoke stack
25 must be within acceptable regulatory levels.

 In the agricultural area, despite the importance of mineral fertilizers in providing mankind with abundant agricultural products, the harm done to the environment has been recognized in recent years. Mineral fertilizers may incurred damages to soils. For example, most nitrogen fertilizers may acidify soils, thereby adversely affecting the growth
30 of plants and other soil organisms. Extensive use of chemical nitrogen fertilizers may also inhibit the activity of natural nitrogen fixing microorganisms, thereby decreasing the natural fertility of soils. The long term use of mineral fertilizers may also cause severe environmental pollution. For example, the loss of nitrogen and phosphate fertilizers due to leaching and soil erosion has led to contamination of soil and ground water, and
35 eutrophication of surface water.

Another type of agricultural waste is manure which, if not stored or disposed of properly, can pose health and environmental threats. For example, it can cause air pollution, i.e., odor and dust; and contamination of surface and ground water with excess nutrients, organic matter, salts, and pathogens. For example, manure contains pathogenic
5 microorganisms, such as *Escherichia coli*, *Salmonella spp.*, and *Shigella spp.*

Overall, cleaning up pollution as a result of a poor waste management strategy has been a complicated and difficult task. The cost for such a task is also astronomical. Thus, there is a need for inexpensive and effective methods to handle the waste generated by the myriad of human activities.

10 The use of biological compositions in pollution control has been proposed for many situations. Biological fertilizers utilizing microorganisms have been proposed as alternatives to mineral fertilizers. Naturally occurring nitrogen fixing microorganisms including bacteria, such as *Rhizobium*, *Azotobacter*, and *Azospirillum*, (See for example, U. S. Patent No. 5,071,462) and fungi, such as *Aspergillus flavus-oryzae*, (See, for example, U.
15 S. Patent No. 4,670,037) have been utilized in biological fertilizers. Naturally occurring microorganisms capable of solubilizing phosphate rock ore or other insoluble phosphates into soluble phosphates have also been utilized in biological fertilizers either separately (e.g., U. S. Patent No. 5,912,398) or in combination with nitrogen fixing microorganisms (e.g., U. S. Patent No. 5,484,464). An approach based on recombinant DNA techniques has
20 been developed to create more effective nitrogen fixing, phosphorus decomposing, and potassium decomposing bacterial strains for use in a biological fertilizer, see, for example, U.S. Patent No. 5,578,486; PCT publication WO 95/09814; Chinese patent publication: CN 1081662A; CN 1082016A; CN 1082017A; CN 1103060A; and CN 1109595A.

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25 documents cited herein is pertinent prior art, or an admission that the cited documents are considered material to the patentability of the claims of the present application. All statements as to the date or representations as to the contents of these documents are based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of these documents.

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3. SUMMARY OF THE INVENTION

The present invention relates to biological compositions useful for the treatment of solid waste. The biological compositions of the invention comprises a plurality of yeast cells which is capable of suppression of growth of pathogenic microorganisms,

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breakdown of undesirable chemicals, such as antibiotics, insecticides and waste chemicals, and reducing the odor of organic waste matters.

In various embodiments, the present invention uses yeasts that are commercially available and/or accessible to the public, such as but not limited to
5 *Saccharomyces cerevisiae*. The yeast cells of the invention are produced by culturing the plurality of yeast cells under activation conditions in the presence of a series of electromagnetic fields, such that the yeast cells become highly efficient in performing certain metabolic functions. Accordingly, the invention also relates to methods for manufacturing the treatment composition comprising culturing the yeast cells under
10 activation conditions, mixing various yeast cell cultures of the present invention, followed by drying the yeast cells and packing the final product.

In particular, the invention encompasses methods for treatment of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the antibiotics in the solid waste, wherein said yeast cells are prepared by
15 culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having specific ranges of frequencies and field strengths. The antibiotics that can be degraded by the yeast cells of invention include are not limited to penicillin, chlortetracycline, oxytetracycline, doxycycline, tetracycline, streptomycin, kanamycin, erythromycin, spiramycin, bacitracin, colistin, chloramphenicol, cephalothin, neomycin and
20 novobiocin.

The invention also encompass methods for treatment of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields
25 having specific ranges of frequencies and field strengths. The undesirable chemicals that can be degraded by the yeast cells of the invention include but are not limited to toluene, ethylbenzene, trichlorophenols, dimethylbenzenes, benzaldehyde, propylaldehyde, nenanthaldehyde, dichlorobenzenes, acetophenone, arsanilic acid, roxarsone, furazolidonum, decoquinate, trichlorophonum, dinitomide, dichlorvos, momocrotophos, dimethoate, DDT and toxaphene. The undesirable chemicals also include organic and
30 inorganic salts such as ammonium compounds, nitrites or nitrates, and phosphates.

The invention further encompasses methods for reducing the odor of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of odorous molecules in the solid waste, wherein said yeast cells
35 are prepared by culturing the yeast cells in an electromagnetic field or a series of

electromagnetic fields having specific ranges of frequencies and field strengths. The odorous molecules include but are not limited to hydrogen sulfide, ammonia, indole, skatol, acetic acid, methylamine, and p-cresol.

The invention further encompasses methods for suppressing the growth of pathogenic bacteria in solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to suppress the growth of pathogenic bacteria in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having specific ranges of frequencies and field strengths. The pathogenic bacteria are selected from the group consisting of *Staphylococcus aureus*, *Diplococcus pneumoniae*, *Bacillus anthracis*, *Mycobacterium tuberculosis*, *Salmonella* species, *E. coli*, *Vibrio* species, *Shigella* species, *Clostridium botulinum*, and *Bacillus aerogenes capsulatus*.

The methods of the invention can be carried out using combinations of the yeast cells in the treatment of solid waste. A biological composition of the invention is added to the solid waste, said biological composition comprising at least one of the following yeast cell components (a) a first yeast cell component comprising a plurality of yeast cells that degrade antibiotics in solid waste; (b) a second yeast cell component comprising a plurality of yeast cells that degrade undesirable chemicals in solid waste; (c) a third yeast cell component comprising a plurality of yeast cells that reduce the amount of odorous molecules in solid waste; and (d) a fourth yeast cell component comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in the solid waste. The amount of time for the treatment can be determined empirically by monitoring the change in levels of the antibiotics, undesirable chemicals, pathogenic bacteria, and malodorous molecules in the solid waste, and can range from several hours, several days, and up to two or more weeks.

The invention further include methods for using the biological compositions of the present invention for the management, storage, processing, recycling or disposal of solid waste.

4. BRIEF DESCRIPTION OF FIGURES

Fig. 1 Activation of yeast cells. 1 yeast cell culture; 2 container; 3 electromagnetic field source.

Fig. 2. Adaptation of yeast cells to a soil type. 4 input electrode; 5 container; 6 electrode; 7 yeast cell culture; 8 electromagnetic field source; 9 temperature controller.

5 DETAILED DESCRIPTION OF THE INVENTION

The present invention provides biological compositions that comprise yeast cells. The present invention also provides methods for manufacturing the biological compositions as well as methods for using the biological compositions.

The biological compositions of the invention are useful for the treatment of solid waste so as to reduce the health risk and its impact on the environment that are often associated with its storage, transportation, processing, recycling and/or disposal. The use of such compositions may lower the overall cost of managing solid waste for a community, a business, or a farm, and make feasible the recycling of certain types of solid waste. As used herein, treatment of a solid waste refers to a process which changes the physical, chemical, or biological character(s) of the solid waste that make the solid waste less objectionable while it is being stored, transported, recycled, handled, or less of a health threat or an environmental threat, than if the solid waste is not treated. The treatment generally renders the waste less hazardous, or makes the solid waste safer to transport, store, handle or recycle.

According to the invention, the biological compositions comprise a plurality of yeast cell components. Each yeast cell component is a population of yeast cells which comprises a plurality of yeast cells that are capable of performing one or more desired functions falling within the following categories: (1) suppression of growth of pathogens, (2) degradation of undesirable chemicals, or (3) reducing the odor of organic materials.

In one embodiment, a biological composition of the invention comprises at least one yeast cell component that can perform a function in one of the three categories. In preferred embodiments, the biological compositions of the invention comprises yeast cell components that provide functions in all three categories. Thus, the preferred biological fertilizer compositions comprise at least three different yeast cell components. It will be understood that different alternative formulations of yeast cell components are contemplated.

As used herein, the term "solid waste" broadly refers to any kind of material that is discarded because it has served its purpose or it is a by-product that is of no use, and that includes physiological waste excreted by humans and animals. The sources of solid

waste include residential, commercial, agricultural and industrial activities. Non-industrial and non-agricultural solid waste such as trash or garbage collected from urban areas, contains discarded food materials or materials used in food preparation, and other assorted dry materials, such as paper, fabric, or plastics. Especially in residential areas and
5 commercial areas which have restaurants and hotels, the predominant type of solid waste, herein referred to as "garbage", comprises mainly decomposable food wastes. Garbage which supports growth of pathogenic organisms and becomes malodorous due to decay, can be efficiently treated by the biological compositions of the invention. The type of solid waste that lends itself to treatment by the biological compositions of the invention has the
10 characteristic of a high organic content.

Another type of solid waste that can be treated by the compositions of the invention is sludge. The term "sludge" as used herein broadly encompasses any solid matter that has settled out of suspension in the course of sewage storage and/or treatment, for example but not limited to, residues in a waste lagoon, residues in an urban sewage
15 treatment plant, or sewage concentrate. The term "sludge" also include semi-solid matters, and mixtures of effluent and sediments. The term thus encompasses sludge having a wide range of viscosity, density, and water content, as well as sludge which has been partially processed or stabilized. Depending on the source, sludge may contain a variety of undesirable chemicals that may have an adverse impact on the environment if it is not
20 properly treated. Sludge is malodorous, and supports growth of pathogenic organisms.

The biological compositions of the invention can also treat waste products produced as a result of agricultural activities. Typically, the waste is produced by animals in operations such as but not limited to ranches, farms, slaughterhouses, and markets. The continuous production and accumulation of large amounts of animal excrement creates a
25 malodorous environment, and poses a health risk to humans and livestock due to the presence of pathogenic microorganisms. Agricultural waste can also contain undesirable chemicals, such as antibiotic feed additives, chemical fertilizers, pesticides, and herbicides, that may pollute the environment if the waste is not managed properly.

The term "animal manure" as used herein broadly encompasses organic
30 material that comprises the feces and urine of feedlot animals with or without accompanying litter such as straw, hay, or bedding, that is traditionally used to fertilize land. Poultry manure includes but are not limited to manure produced by domesticated birds, such as chicken, duck, turkey, goose, quail, squab, ostrich, and the like. Poultry manure include excrement or guano produced by non-domesticated bird species. Cattle manure as used
35 herein encompasses waste from domesticated ruminant mammals, such as dairy cows, or

beef cattle. The term "cattle manure" as used herein is not limited to just cattle but include other animals that graze, and that are kept primarily for their milk, meat, skin, hair, and pelts. Cattle manure includes but are not limited to manure produced by buffalos, bison, yaks, horses, donkeys, mules, sheep, goats, camels, and the like. Cattle manure also include
5 excrement produced by non-domesticated herds. The term "swine manure" as used herein includes but are not limited to manure produced by swines, hogs, pigs, and the like. Other agricultural waste include field crop residues, bagasse, waste from fruit and vegetable packing facilities, waste from animal product packing facilities which include animal carcasses.

10 In various embodiments, the biological compositions of the invention are particularly useful in treating garbage, sludge, and manure.

Municipal waste in many cases is temporarily stored at waste transfer stations. At the transfer station, waste is off-loaded from local collection routes and in some cases sorted according to type. The waste is then loaded onto larger trucks or rail cars for
15 transport to either a municipal waste treatment or disposal facility. Generally, depending on the source of the solid waste, glass, metal, wood, and other inorganic or non-decomposable items are separated from waste of a high organic content through sorting and separating operations. These can be carried out by methods well known in the recycling / garbage disposal industry, such as mechanically, using differences in such physical characteristics of
20 the solid waste as size, and density. Shredding or pulverizing can reduce the size of the waste articles to fine particles, resulting in a uniform mass of material which can be more easily handled, e.g., mixing or transport. Due to the variation of constituents in manure, sludge, or garbage, it may be desirable to subject a sample of a batch of waste material to analysis to determine the amount and type of pathogenic organisms and undesirable
25 chemicals present in the batch.

While the following terms are believed to have well-defined meanings in the art, the following are set forth to facilitate explanation of the invention.

As used herein, the phrase "suppressing the growth of pathogens" refers to a decrease or lack of increase in the number of pathogenic microorganisms present in a
30 sample of solid waste over a period of time, as a result of the presence of the yeast cells of the invention in the sample. It is to be understood that in the absence of the yeast cells, the number of pathogens in the sample would increase naturally. Many such microorganisms cause diseases in humans and animals, and may include bacteria such as *Escherichia* species, *Salmonella* species, *Shigella* species, *Mycobacterium* species, *Staphylococcus*
35 species, *Bacillus* species, *Streptococcus* species, and *Diplococcus* species.

As used herein, the phrase "degradation of undesirable chemicals" refers to biological or biochemical processes which result in the conversion of chemical compounds that are undesirable, e.g., environmental toxins, in solid waste to an inactive form, such as the breakdown of such compounds into lower molecular weight compounds. Antibiotics are commonly present in manure and such compounds are not desired in a fertilizer made from manure because of the potential risk of ingestion by humans, for example, by eating vegetables grown using a fertilizer comprising contaminated organic material, and the possible spread of antibiotic resistance in the environment. Many antibiotics are added to animal feed to protect various farm animals, such as chicken, turkey, and swine, from bacterial and parasitic diseases, and to promote growth. A significant amount of antibiotic feed additive is excreted by the animals, and thus accumulates in manure and sludge. Many kinds of antibiotics have been used in animal operations, such as but not limited to aminoglycosides, tetracyclines, beta-lactams, glycopeptides, and macrolides. Examples of antibiotics approved for use in farms in United States include but are not limited to, bacitracin methylene disalicylate, bacitracin zinc, bambarmycins, oxytetracycline, chlortetracycline, penicillin, tylosin/sulfamethazine, roxarsone, nitrasone, monensin, lasalocid, carbadox, tiamulin, hygromycin B, nystatin, novobiocin, sulfadimethoxine, ormetoprim, lincomycin, fenbendazole, and virginiamycin. The presence and quantity of such antibiotics in a composition can be determined by any methods known in the art, for example, high performance liquid chromatography (HPLC).

As used herein, the phrase "reducing the odor of organic materials" refers to a process which results in a lower concentration of one or more odorous compounds in the organic materials present in solid waste. Odorous compounds, such as but not limited to hydrogen sulfide, ammonia, indole, skatole (i.e, 3-methyl-1H-indole), p-cresol, and organic acids, are known to contribute to the malodorous quality of solid waste. The concentration of such malodorous compounds in, for example, poultry manure or in a sample of air in contact with the manure can be determined by any method well known in the art, including but not limited to gas chromatography, or mass spectrometry. Odor is a perception of smell by an organism with olfactory organs. A reduction of the intensity of the odor associated with solid waste can be determined subjectively. Various methods and techniques are known to measure the intensity of an odor. One subjective measurement of odor intensity is to measure the dilution necessary so that the odor is imperceptible or doubtful to a human or animal test panel. Alternatively, a recognition threshold may also be used which is a higher concentration at which the character of the odor is recognized. Any methods and

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techniques for objectively or subjectively determine the intensity of an odor can be used to monitor the performance of the compositions and methods of the invention.

The inventor discovered that, under certain culture conditions, yeasts can be activated to become highly efficient in certain metabolic functions which result in an ability of the activated yeasts to suppress growth of pathogens, degrade undesirable chemicals, or reduce the odor of organic materials.

According to the invention, a yeast cell component of the biological fertilizer composition is produced by culturing a plurality of yeast cells in an appropriate culture medium in the presence of an alternating electromagnetic field or multiple alternating electromagnetic fields in series over a period of time. The culturing process allows yeast spores to germinate, yeast cells to grow and divide, and can be performed as a batch process or a continuous process. As used herein, the terms "alternating electromagnetic field", "electromagnetic field" or "EM field" are synonymous. An electromagnetic field useful in the invention can be generated by various means well known in the art. A schematic illustration of exemplary setups are depicted respectively in Fig. 1. An electromagnetic field of a desired frequency and a desired field strength is generated by an electromagnetic wave source (3) which comprises one or more signal generators that are capable of generating electromagnetic waves, preferably sinusoidal waves, and preferably in the frequency range of 30 MHz - 3000 MHz. Such signal generators are well known in the art. Signal generators capable of generating signal with a narrower frequency range can also be used. If desirable, a signal amplifier can also be used to increase the output signal, and thus the strength of the EM field.

The electromagnetic field can be applied to the culture by a variety of means including placing the yeast cells in close proximity to a signal emitter connected to a source of electromagnetic waves. In one embodiment, the electromagnetic field is applied by signal emitters in the form of electrodes that are submerged in a culture of yeast cells (1). In a preferred embodiment, one of the electrodes is a metal plate, and the other electrode comprises a plurality of wires configured inside the container (2) so that the energy of the electromagnetic field can be evenly distributed in the culture. The number of electrode wires used depends on both the volume of the culture and the diameter of the wire. For example, for a culture having a volume of 5000 ml, one electrode wire having a diameter of between 0.1 to 1.2 mm can be used for each 100 ml of culture; for a culture having a volume greater than 1000 l, one electrode wire having a diameter of between 3 to 30 mm can be used for each 1000 l of culture. See Figure 1.

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Without being bound by any theory or mechanism, the inventor believes that the culture conditions activate and/or enhance the expression of a gene or a set of genes in a yeast cell such that the cell becomes more efficient in performing certain metabolic activities which lead to the respective desired results.

- 5 In various embodiments, yeasts of the genera of *Saccharomyces*, *Schizosaccharomyces*, *Sporobolomyces*, *Torulopsis*, *Trichosporon*, *Wickerhamia*, *Ashbya*, *Blastomyces*, *Candida*, *Citeromyces*, *Crebrothecium*, *Cryptococcus*, *Debaryomyces*, *Endomycopsis*, *Geotrichum*, *Hansenula*, *Kloeckera*, *Lipomyces*, *Pichia*, *Rhodospiridium*, and *Rhodotorula* can be used in the invention.
- 10 Non-limiting examples of yeast strains include *Saccharomyces cerevisiae* Hansen, ACCC2034, ACCC2035, ACCC2036, ACCC2037, ACCC2038, ACCC2039, ACCC2040, ACCC2041, ACCC2042, AS2.1, AS2.4, AS2.11, AS2.14, AS2.16, AS2.56, AS2.69, AS2.70, AS2.93, AS2.98, AS2.101, AS2.109, AS2.110, AS2.112, AS2.139, AS2.173, AS2.174, AS2.182, AS2.196, AS2.242, AS2.336, AS2.346, AS2.369, AS2.374, AS2.375, AS2.379, AS2.380, AS2.382, AS2.390, AS2.393, AS2.395, AS2.396, AS2.397, AS2.398, AS2.399, AS2.400, AS2.406, AS2.408, AS2.409, AS2.413, AS2.414, AS2.415, AS2.416, AS2.422, AS2.423, AS2.430, AS2.431, AS2.432, AS2.451, AS2.452, AS2.453, AS2.458, AS2.460, AS2.463, AS2.467, AS2.486, AS2.501, AS2.502, AS2.503, AS2.504, AS2.516, AS2.535, AS2.536, AS2.558, AS2.560, AS2.561, AS2.562, AS2.576, AS2.593, AS2.594, AS2.614, AS2.620, AS2.628, AS2.631, AS2.666, AS2.982, AS2.1190, AS2.1364, AS2.1396, IFFI 1001, IFFI 1002, IFFI 1005, IFFI 1006, IFFI 1008, IFFI 1009, IFFI 1010, IFFI 1012, IFFI 1021, IFFI 1027, IFFI 1037, IFFI 1042, IFFI 1043, IFFI 1045, IFFI 1048, IFFI 1049, IFFI 1050, IFFI 1052, IFFI 1059, IFFI 1060, IFFI 1063, IFFI 1202, IFFI 1203, IFFI 1206, IFFI 1209, IFFI 1210, IFFI 1211, IFFI 1212, IFFI 1213, IFFI 1215, IFFI 1220, IFFI 1221, IFFI 1224, IFFI 1247, IFFI 1248, IFFI 1251, IFFI 1270, IFFI 1277, IFFI 1287, IFFI 1289, IFFI 1290, IFFI 1291, IFFI 1292, IFFI 1293, IFFI 1297, IFFI 1300, IFFI 1301, IFFI 1302, IFFI 1307, IFFI 1308, IFFI 1309, IFFI 1310, IFFI 1311, IFFI 1331, IFFI 1335, IFFI 1336, IFFI 1337, IFFI 1338, IFFI 1339, IFFI 1340, IFFI 1345, IFFI 1348, IFFI 1396, IFFI 1397, IFFI 1399, IFFI 1411, IFFI 1413; *Saccharomyces cerevisiae* Hansen Var. ellipsoideus (Hansen) Dekker, ACCC2043, AS2.2, AS2.3, AS2.8, AS2.53, AS2.163, AS2.168, AS2.483, AS2.541, AS2.559, AS2.606, AS2.607, AS2.611, AS2.612; *Saccharomyces chevalieri* Guillermond, AS2.131, AS2.213; *Saccharomyces delbrueckii*, AS2.285; *Saccharomyces delbrueckii* Lindner var. mongolicus Lodder et van Rij, AS2.209, AS2.1157; *Saccharomyces exiguus* Hansen, AS2.349, AS2.1158; *Saccharomyces fermentati* (Saito) Lodder et van Rij, AS2.286, AS2.343; *Saccharomyces logos* van laer et
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- Denamur ex Jorgensen, AS2.156, AS2.327, AS2.335; *Saccharomyces mellis* Lodder et Kreger Van Rij, AS2.195; *Saccharomyces microellipsoides* Osterwalder, AS2.699; *Saccharomyces oviformis* Osterwalder, AS2.100; *Saccharomyces rosei* (Guilliermond) Lodder et kreger van Rij, AS2.287; *Saccharomyces rouxii* Boultroux, AS2.178, AS2.180, 5 AS2.370, AS2.371; *Saccharomyces sake* Yabe, ACCC2045; *Candida arborea*, AS2.566; *Candida Krusei* (Castellani) Berkhout, AS2.1045; *Candida lambica* (Lindner et Genoud) van.Uden et Buckley, AS2.1182; *Candida lipolytica* (Harrison) Diddens et Lodder, AS2.1207, AS2.1216, AS2.1220, AS2.1379, AS2.1398, AS2.1399, AS2.1400; *Candida parapsilosis* (Ashford) Langeron et Talice, AS2.590; *Candida parapsilosis* (Ashford) et 10 Talice Var. *intermedia* Van Rij et Verona, AS2.491; *Candida pulcherriman* (Lindner) Windisch, AS2.492; *Candida rugosa* (Anderson) Diddens et Loddeer, AS2.511, AS2.1367, AS2.1369, AS2.1372, AS2.1373, AS2.1377, AS2.1378, AS2.1384; *Candida tropicalis* (Castellani) Berkout, ACCC2004, ACCC2005, ACCC2006, AS2.164, AS2.402, AS2.564, AS2.565, AS2.567, AS2.568, AS2.617, AS2.1387; *Candida utilis* Henneberg 15 Lodder et Kreger Van Rij, AS2.120, AS2.281, AS2.1180; *Crebrothecium ashbyii* (Guilliermond) Routein, AS2.481, AS2.482, AS2.1197; *Geotrichum candidum* Link, ACCC2016, AS2.361, AS2.498, AS2.616, AS2.1035, AS2.1062, AS2.1080, AS2.1132, AS2.1175, AS2.1183; *Hansenula anomala* (Hansen) H et P sydow, ACCC2018, AS2.294, AS2.295, AS2.296, AS2.297, AS2.298, AS2.299, AS2.300, AS2.302, AS2.338, AS2.339, 20 AS2.340, AS2.341, AS2.470, AS2.592, AS2.641, AS2.642, AS2.635, AS2.782, AS2.794; *Hansenula arabitolgens* Fang, AS2.887; *Hansenula jadinii* Wickerham, ACCC2019; *Hansenula saturnus* (Klocker) H et P sydow, ACCC2020; *Hansenula schneegii* (Weber) Dekker, AS2.304; *Hansenula subpelliculosa* Bedford, AS2.738, AS2.740, AS2.760, AS2.761, AS2.770, AS2.783, AS2.790, AS2.798, AS2.866; *Kloeckera apiculata* (Reess 25 emend. Klocker) Janke, ACCC2021, ACCC2022, ACCC2023, AS2.197, AS2.496, AS2.711, AS2.714; *Lipomyces starkeyi* Lodder et van Rij, ACCC2024, AS2.1390; *Pichia farinosa* (Lindner) Hansen, ACCC2025, ACCC2026, AS2.86, AS2.87, AS2.705, AS2.803; *Pichia membranaefaciens* Hansen, ACCC2027, AS2.89, AS2.661, AS2.1039; *Rhodosporidium toruloides* Banno, ACCC2028; *Rhodotorula glutinis* (Fresenius) Harrison, 30 ACCC2029, AS2.280, ACCC2030, AS2.102, AS2.107, AS2.278, AS2.499, AS2.694, AS2.703, AS2.704, AS2.1146; *Rhodotorula minuta* (Saito) Harrison, AS2.277; *Rhodotorula rubar* (Demme) Lodder, ACCC2031, AS2.21, AS2.22, AS2.103, AS2.105, AS2.108, AS2.140, AS2.166, AS2.167, AS2.272, AS2.279, AS2.282; *Saccharomyces carlsbergensis* Hansen, ACCC2032, ACCC2033, AS2.113, AS2.116, AS2.118, AS2.121, 35 AS2.132, AS2.162, AS2.189, AS2.200, AS2.216, AS2.265, AS2.377, AS2.417, AS2.420,

- AS2.440, AS2.441, AS2.443, AS2.444, AS2.459, AS2.595, AS2.605, AS2.638, AS2.742, AS2.745, AS2.748, AS2.1042; *Saccharomyces uvarum* Beijer, IFFI 1023, IFFI 1032, IFFI 1036, IFFI 1044, IFFI 1072, IFFI 1205, IFFI 1207; *Saccharomyces willianus* Saccardo, AS2.5, AS2.7, AS2.119, AS2.152, AS2.293, AS2.381, AS2.392, AS2.434, AS2.614,
- 5 AS2.1189; *Saccharomyces* sp., AS2.311; *Saccharomyces ludwigii* Hansen, ACCC2044, AS2.243, AS2.508; *Saccharomyces sinenses* Yue, AS2.1395; *Schizosaccharomyces octosporus* Beijerinck, ACCC 2046, AS2.1148; *Schizosaccharomyces pombe* Linder, ACCC2047, ACCC2048, AS2.248, AS2.249, AS2.255, AS2.257, AS2.259, AS2.260, AS2.274, AS2.994, AS2.1043, AS2.1149, AS2.1178, IFFI 1056; *Sporobolomyces roseus*
- 10 Kluyver et van Niel, ACCC 2049, ACCC 2050, AS2.619, AS2.962, AS2.1036, ACCC2051, AS2.261, AS2.262; *Torulopsis candida* (Saito) Lodder, ACCC2052, AS2.270; *Torulopsis famta* (Harrison) Lodder et van Rij, ACCC2053, AS2.685; *Torulopsis globosa* (Olson et Hammer) Lodder et van Rij, ACCC2054, AS2.202; *Torulopsis inconspicua* Lodder et van Rij, AS2.75; *Trichosporon behrendii* Lodder et Kreger van Rij, ACCC2055, AS2.1193;
- 15 *Trichosporon capitatum* Diddens et Lodder, ACCC2056, AS2.1385; *Trichosporon cutaneum* (de Beurm et al.) Ota, ACCC2057, AS2.25, AS2.570, AS2.571, AS2.1374; *Wickerhamia fluoresens* (Soneda) Soneda, ACCC2058, AS2.1388.

Certain yeast species that can be activated or induced according to the present invention and are included in the present invention are known to be pathogenic to

20 human and/or other living organisms, for example, *Ashbya gossypii*; *Blastomyces dermatitidis*; *Candida albicans*; *Candida parakrusei*; *Candida tropicalis*; *Citeromyces matritensis*; *Crebrothecium ashbyii*; *Cryptococcus laurentii*; *Cryptococcus neoformans*; *Debaryomyces hansenii*; *Debaryomyces klockeri*; *Debaryomyces* sp.; *Endomycopsis fibuligera*. Under certain circumstances, it may be less preferable to use such pathogenic

25 yeasts in the biological compositions of the invention, for example, if such use is in an open field, it may endanger the health of human and/or other living organisms.

Yeasts of the *Saccharomyces* genus are generally preferred. Among strains of *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* Hansen is a preferred strain. The most preferred strains of yeast are *Saccharomyces cerevisiae* strains having accession

30 numbers AS2.504, AS2.558, AS2.413, AS2.397, AS2.69, AS2.109, AS2.607, AS2.516, AS2.561, AS2.422, AS2.393, AS2.631, AS2.982, AS2.560, AS2.467, AS2.415, AS2.375, AS2.628, AS2.1190, AS2.562, AS2.463, AS2.409, AS2.379, AS2.666, AS2.631, AS2.182, AS2.431, AS2.606, AS2.53, AS2.611, AS2.414, AS2.576, AS2.483, IFFI 1211, IFFI 1293, IFFI 1308, IFFI 1210, IFFI 1213, IFFI 1307, IFFI 1206, IFFI 1052, IFFI 1301, IFFI 1291,

35 IFFI 1202, IFFI 1021, IFFI 1059, IFFI 1052, IFFI 1441, IFFI 1008, IFFI 1220, IFFI 1302,

and IFFI 1023 as deposited at the China General Microbiological Culture Collection Center (CGMCC).

Generally, yeast strains useful for the invention can be obtained from private or public laboratory cultures, or publically accessible culture deposits, such as the American
5 Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 and the China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture Collection of Microorganisms, Institute of Microbiology, Chinese Academy of Sciences, Haidian, P.O. Box 2714, Beijing, 100080, China.

Although it is preferred, the preparation of the yeast cell components of the
10 invention is not limited to starting with a pure strain of yeast. Each yeast cell component may be produced by culturing a mixture of yeast cells of different species or strains. The constituents of a yeast cell component can be determined by standard yeast identification techniques well known in the art.

The ability and efficiency of the activated yeasts to perform a desired
15 function before or after culturing under the conditions of the invention can readily be tested by methods known in the art. For example, HPLC or mass spectrometry can be used for detecting and analyzing various organic molecules in a sample of solid waste. Microbiological methods well known in the art can be used for detecting and counting the number of viable microorganisms and the total number of microorganisms in a sample.

20 When treating organic manure that has a relatively high bacteria count, the biological composition can be formulated to comprise largely yeast cells that suppress bacterial growth. When the biological composition is to be used to treat solid waste that is contaminated with undesirable chemicals, the biological composition can be formulated to comprise mostly yeast cells that degrade undesirable chemicals. Thus, the biological
25 compositions have utility in many types of situations encountered in municipal, commercial, agricultural, and industrial establishments. The invention can also be used domestically, especially in rural areas.

The biological compositions of the invention can be applied directly to the solid waste. As known to those skilled in the relevant art, many methods and appliances
30 may be used to mix the yeasts with the solid waste. In one embodiment, a culture broth of the yeasts of the present invention are added directly to the solid waste to be treated. In another embodiment, dried powders of the yeasts of the present invention are mixed with the solid waste to which water is added at a later time. The biological compositions may be applied to and mixed with the solid waste by spreaders, sprayers, and other mechanized
35 means which may be automated. The amount of biological composition used depends in

part on the circumstances and the type of solid waste, and can be determined empirically. However, to achieve an effective treatment, it is desired to use from about 300 to 600g dry weight (less than 10% moisture) of the biological composition per cubic meter of solid waste. The yeast cells are first mixed with water in the ratio of about 1000 g yeasts (dry weight) to about 30 liters, and then incubated for 12 to 24 hours, prior to application to the solid waste. The benefit of the treatment, e.g., reduction of odor or bacteria count, takes effect about 24 to 72 hours after the application. Although not necessary, the biological compositions of the invention can also be used in conjunction or in rotation with other types of deodorants, disinfectants, and detoxification agents.

Described respectively in Sections 5.1 - 5.4 are the yeast cell components used for degradation of antibiotics, pathogen suppression, degradation of undesirable chemicals, and reduction of odor. Methods for preparing each yeast cell components are described. Section 5.6 describes the manufacture of the biological compositions of the invention. In various embodiments of the invention, standard techniques for handling, transferring, and storing yeasts are used. Although it is not necessary, sterile conditions or clean environments are desirable when carrying out the manufacturing processes of the invention.

5.1. YEAST CELLS THAT DEGRADE ANTIBIOTICS

The present invention provides yeast cells that are capable of degrading antibiotics that are typically found in manures and sludge.

According to the invention, the ability of yeast cells to degrade antibiotics is activated or enhanced by culturing the yeast cells in the presence of an electromagnetic field in an appropriate culture medium. The resulting yeast cells can be used as a component in the biological solid waste treatment compositions of the invention.

The frequency of the electromagnetic field for activating or enhancing the ability of yeasts to degrade antibiotics can generally be found in the range of 70 MHz to 600 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their enhanced ability to decompose one or more types of antibiotics by methods well known in the art. Antibiotics degraded by the yeasts of the invention include but are not limited to molecules within the families of beta-lactams, tetracyclines, polypeptides, glycopeptides, aminoglycosides, and macrolides.

The method of the invention for making antibiotics-degrading yeasts is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose,

fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies
 5 between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate,
 10 and like ions. Non-limiting examples of nutrient inorganic salts are $(\text{NH}_4)_2\text{HPO}_4$, CaCO_3 , MgSO_4 , NaCl , and CaSO_4 .

Table 1: Composition for a culture medium for yeasts that degrade antibiotics

15	Medium Composition	Quantity
	Manure or sludge	8.0g, dry weight, >120 mesh
	NaCl	0.2g
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
20	$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	0.5g
	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.2g
	Peptone	1.5g
	K_2HPO_4	0.5g
25	Extract containing antibiotics (≥ 100 ug/ml)	600ml
	Autoclaved water	400ml

The extract containing antibiotics is prepared by dispersing and incubating
 30 500g of fresh waste, e.g., manures, sludge, in about 600ml of warm water (at 35-40°C) for 24 hours at 30-37°C, and filtering the fluid to remove particulate matters. If the extract contains only negligible amount of a particular antibiotics, an appropriate amount of the antibiotics can be added into the extract.

It should be noted that the composition of the media provided in Table 1 is
 35 not intended to be limiting. Various modifications of the culture medium may be made by

those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2 - 10^5 cell/ml, preferably 3×10^2 -
5 10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

10 The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 70.000 to 100.000 MHz and 410.000 to 620.000 MHz, preferably. The field strength of the EM field(s) is in the range of 40 to 250 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different
15 frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different
20 EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the
25 presence of the EM field or EM fields for a total of about 144 - 384 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of 8.5-85mV/cm, usually at about 50 mV/cm, is used. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased
30 to a higher level in the range of 150-250 mV, usually to about 200 mV. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m³, preferably 0.4 mol/m³. The oxygen level can be controlled by any

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conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C to 4°C. The recovered yeast cells may also be dried and stored in powder form.

To determine the activity of the activated yeast cells towards an antibiotic compound, methods well known in the art, such as HPLC, can be used to measure the amounts of the antibiotic compound in a test sample at various time point and under different incubation conditions. For example, a sample containing known concentration of an antibiotic (up to 100 mg per liter) is prepared. Then, 0.1 ml of activated and unactivated yeasts (at least 10^7 cells/ml) are added to the 100 liter samples containing the antibiotics, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the extracts are determined and compared by performing HPLC on the samples.

The method is generally applicable to many type of antibiotics. In specific embodiments, methods that are optimized for a particular type of antibiotics are described, infra.

Yeast cell component that decomposes penicillins

In a specific embodiment, a method for producing yeast cells that decompose penicillins, e.g., penicillin G and Cloxacillin, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 100.000 MHz, including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, and 100 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.399 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 77 MHz at 48 mV/cm for 15 h; 83 MHz at 48 mV/cm for 15 h; 90 MHz at 48 mV/cm for 15 h; 96 MHz at 48 mV/cm for 15 h; 77 MHz at 200 mV/cm for 30 h; 83 MHz at 200 mV/cm for 30 h; 90 MHz at 200 mV/cm for 30 h; 96 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards penicillin was determined by measuring the amounts of penicillin the activated yeast cells can degrade. Two 100 liter samples each containing a penicillin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of

antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of penicillin in the sample with the activated yeast cells was reduced by more than 56.5%.

5 Yeast cell component that decomposes chlortetracycline

In a specific embodiment, a method for producing yeast cells that decompose chlortetracycline, e.g., aureomycin, chlortetracycline hydrochloridum, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 10 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.748 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 73 MHz at 48 mV/cm for 15 h; 88 MHz at 48 15 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards chlorotetracycline was determined by measuring the amounts of chlorotetracycline the activated yeast cells can degrade. Two 100 liter samples each containing a chlorotetracycline concentration of 20 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the two samples. Comparing to samples with 25 unactivated yeast cells, the amount of chlorotetracycline in the samples with the activated yeast cells was reduced by more than 62.3%.

Yeast cell component that decomposes oxytetracycline

30 In a specific embodiment, a method for producing yeast cells that decompose oxytetracycline, e.g., oxytetracycline hydrochloridum, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, Yeast cells of 35 *Saccharomyces cerevisiae* strain AS2.101 are cultured at about 25-30°C in a culture

medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 74 MHz at 48 mV/cm for 15 h; 88 MHz at 44 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 74 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

5 The activity of the activated yeast cells towards oxytetracycline was determined by measuring the amounts of oxytetracycline the activated yeast cells can degrade. Two 100 liter samples each containing an oxytetracycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and
10 incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with
Comparing to samples unactivated yeast cells, the amount of oxytetracycline in the samples with the activated yeast cells was reduced by more than 65.5%.

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Yeast cell component that decomposes doxycycline

In a specific embodiment, a method for producing yeast cells that decompose doxycycline is provided. The frequencies of the EM field(s) used to activate the yeast cells
20 are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.417 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 71 MHz at 48 mV/cm for 15 h; 73 MHz at 48
25 mV/cm for 15 h; 77 MHz at 48 mV/cm for 15 h; 88 MHz at 48 mV/cm for 15 h; 71 MHz at 200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 77 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards doxycycline was determined by measuring the amounts of doxycycline the activated yeast cells can degrade. Two 100
30 liter samples each containing a doxycycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples are determined and compared by
35 performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the

amount of doxycycline in the samples with the activated yeast cells was reduced by more than 54.9%.

Yeast cell component that decomposes tetracycline

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In a specific embodiment, a method for producing yeast cells that decompose tetracycline is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.70 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 75 MHz at 48 mV/cm for 15 h; 82 MHz at 48 mV/cm for 15 h; 85 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 75 MHz at 200 mV/cm for 30 h; 82 MHz at 200 mV/cm for 30 h; 85 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards tetracycline was determined by measuring the amounts of tetracycline the activated yeast cells can degrade. Two 100 liter samples each containing a tetracycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10⁷ cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples are determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of tetracycline in the samples with the activated yeast cells was reduced by more than 67.6%.

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Yeast cell component that decomposes streptomycin

In a specific embodiment, a method for producing yeast cells that decompose streptomycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.441 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 73 MHz at 48 mV/cm for 15 h; 80 MHz at 48 mV/cm for 15 h; 96 MHz at 48 mV/cm for 15 h; 70 MHz at

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200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 80 MHz at 200 mV/cm for 30 h; 96 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards streptomycin was determined by measuring the amounts of streptomycin the activated yeast cells can degrade. Two 100
 5 liter samples each containing a streptomycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by
 10 performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of streptomycin in the samples with the activated yeast cells was reduced by more than 77.8%.

Yeast cell component that decomposes kanamycin

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In a specific embodiment, a method for producing yeast cells that decompose kanamycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and
 20 98 MHz. For example, east cells of *Saccharomyces cerevisiae* strain AS2.336 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 71 MHz at 48 mV/cm for 15 h; 78 MHz at 48 mV/cm for 15 h; 86 MHz at 48 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 71 MHz at 200 mV/cm for 30 h; 78 MHz at 200 mV/cm for 30 h; 86 MHz at 200 mV/cm for 30 h; 98
 25 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards kanamycin was determined by measuring the amounts of kanamycin the activated yeast cells can degrade. Two 100 liter samples each containing a kanamycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7
 30 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of kanamycin in the samples with the activated yeast cells was reduced by more than 68.7%.

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Yeast cell component that decomposes erythromycin

In a specific embodiment, a method for producing yeast cells that decompose erythromycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.422 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 73 MHz at 48 mV/cm for 15 h; 79 MHz at 48 mV/cm for 15 h; 88 MHz at 48 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 73 MHz at 200 mV/cm for 30 h; 79 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards erythromycin was determined by measuring the amounts of erythromycin the activated yeast cells can degrade. Two 100 liter samples each containing a erythromycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of erythromycin in the samples with the activated yeast cells was reduced by more than 72.7%.

Yeast cell component that decomposes spiramycin

In a specific embodiment, a method for producing yeast cells that decompose spiramycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.620 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 77 MHz at 48 mV/cm for 15 h; 84 MHz at 48 mV/cm for 15 h; 93 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 77 MHz at 200 mV/cm for 30 h; 84 MHz at 200 mV/cm for 30 h; 93 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards spiramycin was determined by measuring the amounts of spiramycin the activated yeast cells can degrade. Two 100 liter samples each containing a spiramycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of spiramycin in the samples with the activated yeast cells was reduced by more than 66.8%.

Yeast cell component that decomposes bacitracin

In a specific embodiment, a method for producing yeast cells that decompose bacitracin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.486 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 75 MHz at 48 mV/cm for 15 h; 78 MHz at 48 mV/cm for 15 h; 81 MHz at 48 mV/cm for 15 h; 95 MHz at 48 mV/cm for 15 h; 75 MHz at 200 mV/cm for 30 h; 78 MHz at 200 mV/cm for 30 h; 81 MHz at 200 mV/cm for 30 h; 95 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards bacitracin was determined by measuring the amounts of bacitracin the activated yeast cells can degrade. Two 100 liter samples each containing a bacitracin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of bacitracin in the samples with the activated yeast cells was reduced by more than 71.6%.

Yeast cell component that decomposes colistin

In a specific embodiment, a method for producing yeast cells that decompose colistin or colistin sulfate is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.611 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 433 MHz at 85 mV/cm for 12 h; 440 MHz at 85 mV/cm for 12 h; 446 MHz at 85 mV/cm for 12 h; 457 MHz at 85 mV/cm for 12 h; 433 MHz at 204 mV/cm for 24 h; 440 MHz at 204 mV/cm for 24 h; 446 MHz at 204 mV/cm for 24 h; 457 MHz at 204 mV/cm for 24 h.

The activity of the activated yeast cells towards colistin was determined by measuring the amounts of colistin the activated yeast cells can degrade. Two 100 liter samples each containing a colistin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of colistin in the samples with the activated yeast cells was reduced by more than 71.6%.

Yeast cell component that decomposes chloramphenicol

In a specific embodiment, a method for producing yeast cells that decompose chloramphenicol and salts such as chloromycetin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.371 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 419 MHz at 85 mV/cm for 12 hr; 425 MHz at 85 mV/cm for 12 h; 433 MHz at 85 mV/cm for 12 h; 462 MHz at 85 mV/cm for 12 h; 419 MHz at 183 mV/cm for 24 h; 425 MHz at 183 mV/cm for 24 h; 433 MHz at 183 mV/cm for 24 h; 462 MHz at 183 mV/cm for 24 h.

The activity of the activated yeast cells towards chloramphenicol was determined by measuring the amounts of chloramphenicol the activated yeast cells can degrade. Two 100 liter samples each containing a chloramphenicol concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of chloramphenicol in the samples with the activated yeast cells was reduced by more than 58.6%.

Yeast cell component that decomposes cephalosporins

In a specific embodiment, a method for producing yeast cells that decompose cephalosporins, e.g., cephalothin, cephaloridine, cephaloglyin, cephalolexin, and cephazoline, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.559 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 434 MHz at 85 mV/cm for 12 h, 441 MHz at 85 mV/cm for 12 h, 450 MHz at 85 mV/cm for 12 h, 458 MHz at 85 mV/cm for 12 h; 434 MHz at 198 mV/cm for 24 h, 441 MHz at 198 mV/cm for 24 h, 450 MHz at 198 mV/cm for 24 h, 458 MHz at 198 mV/cm for 24 h.

The activity of the activated yeast cells towards cephalosporins was determined by measuring the amounts of cephalothin the activated yeast cells can degrade. Two 100 liter samples each containing a cephalothin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of cephalothins in the samples with the activated yeast cells was reduced by more than 75.5%.

Yeast cell component that decomposes neomycin

In a specific embodiment, a method for producing yeast cells that decompose neomycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are
5 in the range of 550.000 to 620.000 MHz, including but not limited to 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, and 575 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.182 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 557 MHz at 85 mV/cm for 12 h, 564 MHz at 85 mV/cm
10 for 12 h, 568 MHz at 85 mV/cm for 12 h, 574 MHz at 85 mV/cm for 12 h; 557 MHz at 231 mV/cm for 24 h, 564 MHz at 231 mV/cm for 24 h, 568 MHz at 231 mV/cm for 24 h, 574 MHz at 231 mV/cm for 24 h.

The activity of the activated yeast cells towards neomycin was determined by measuring the amounts of neomycin the activated yeast cells can degrade. Two 100 liter
15 samples each containing a neomycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10⁷ cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing
20 HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of neomycin in the samples with the activated yeast cells was reduced by more than 67.7%.

Yeast cell component that decomposes novobiocin

25 In a specific embodiment, a method for producing yeast cells that decompose novobiocin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 550.000 to 620.000 MHz, including but not limited to 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, and 610 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.112 were
30 cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 594 MHz at 85 mV/cm for 12 h, 599 MHz at 85 mV/cm for 12 h, 602 MHz at 85 mV/cm for 12 h, 608 MHz at 85 mV/cm for 12 h; 594 MHz at 231 mV/cm for 24 h, 599 MHz at 231 mV/cm for 24 h, 602 MHz at 231 mV/cm for 24 h, 608 MHz at 231 mV/cm for 24 h.

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The activity of the activated yeast cells towards novobiocin was determined by measuring the amounts of novobiocin the activated yeast cells can degrade. Two 100 liter samples each containing a novobiocin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of novobiocin in the samples with the activated yeast cells was reduced by more than 69.5%.

5.2. YEAST CELL COMPONENTS THAT DECOMPOSE UNDESIRABLE CHEMICALS

The present invention further provides yeast cells that are capable of degrading chemicals that are typically found in solid waste.

According to the invention, the ability of yeast cells to degrade undesirable chemicals is activated or enhanced by culturing the yeast cells in the presence of an electromagnetic field in an appropriate culture medium. The resulting yeast cells can be used as a component in the biological waste treatment compositions of the invention.

The frequency of the electromagnetic field for activating or enhancing the ability of yeasts to degrade undesirable chemicals can generally be found in the range of 30 to 280 MHz, 410 to 440 MHz, 660 to 690 MHz, 1400 to 1435 MHz, and 1980 to 2210 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their enhanced ability to decompose one or more types of chemicals by methods well known in the art. Undesirable chemicals degraded by the yeasts of the invention include but are not limited to herbicides, pesticides, and fertilizer-related pollutants.

The method of the invention for making chemical-degrading yeasts is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5%

and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(\text{NH}_4)_2\text{HPO}_4$, CaCO_3 , MgSO_4 , NaCl , and CaSO_4 .

Table 2: Composition for a culture medium for yeasts that degrade chemicals

Medium Composition	Quantity
Manure or sludge	8.0g, dry weight, >120 mesh
NaCl	0.2g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	0.5g
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.2g
Peptone	1.5g
K_2HPO_4	0.5g
Extract containing chemicals (≥ 100 ug/ml)	600ml
Autoclaved water	400ml

The extract for the culture medium is prepared by incubating 500g of fresh waste, e.g., manures, sludge, and/or garbage, in about 600ml of warm water (at 35-40°C) for 24 hours at 30-37°C, and filtering the fluid to remove particulate matters. If the extract contains low amount of the particular chemicals, an appropriate amount of the chemical can be added into the extract.

It should be noted that the composition of the media provided in Table 2 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2 - 10^5 cell/ml, preferably 3×10^2 - 10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series

of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can
5 each have a frequency in the ranges of 30.000 to 100.000, 70.000 to 280.000, 410.000 to 430.000, 660.000 to 680.000 and 1980.000 to 2210.000 MHz. The field strength of the EM field(s) is in the range of 40 to 250 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated
10 ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

15 Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 90 - 480 hours.

20 For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of about 8 to about 300 mV/cm is used. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is
25 between 0.025 to 0.8 mol/m³, preferably 0.4 mol/m³. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C
30 to 4°C. The recovered yeast cells may also be dried and stored in powder.

To determine the activity of the activated yeast cells towards a chemical compound, methods well known in the art, such as HPLC, can be used to measure the amounts of the compound in a test sample at various time point and under different incubation conditions. For example, a sample containing a known concentration of a
35 chemical compound (up to 100 mg per liter) is prepared. Then, 0.1 ml of activated and

unactivated yeasts (at least 10^7 cells/ml) were added to the 100 liter samples containing the compound, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of compound remaining in the extracts are determined and compared by performing HPLC on the samples.

- 5 The method is generally applicable to many classes of chemicals. In specific embodiments, method that are optimized for a particular class of chemicals are described, infra.

Yeast cell component that decomposes aromatic compounds

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- In specific embodiment, method for producing yeast cells that decompose trichlorophenol, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 30.000 to 100.000 MHz, or preferably 52 to 98 MHz including but not limited to 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 15 94, 96, and 98 MHz. Yeast cells of *Saccharomyces cerevisiae* strain IFFI1411 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 6 EM fields in the order stated: 82 MHz at 82 mv/cm for 25 h; 90 MHz at 82 mv/cm for 25 h; 98 MHz at 82mv/cm for 25 h; 82 MHz at 274 mv/cm for 32 h; 90 MHz at 274 mv/cm for 32 h; 98 MHz at 274 mv/cm for 25 h.

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- The activity of the activated yeast cells towards trichlorophenol was determined by measuring the amounts of the compound the activated yeast cells can degrade. Two 100 liter samples each containing the compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and 25 incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of the compound remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of tripchlorophenol in the samples with the activated yeast cells was reduced by more than 56.4%.

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- In another specific embodiment, method for producing yeast cells that decompose toluene or ethylbenzene, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 52.000 to 98.000 MHz, including but not limited to 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.56 were 35 cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a

series of 4 EM fields in the order stated: 76 MHz at 89 mV/cm for 20 h; 80 MHz at 89 mV/cm for 200 h; 86 MHz at 89 mV/cm for 20 h; and 96 MHz at 89 mV/cm for 20 h.

The activity of the activated yeast cells towards toluene or ethylbenzene was determined by measuring the amounts of the compounds the activated yeast cells can
5 degrade. Two 100 liter samples each containing the compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of the compound remaining in the samples were
10 determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of toluene in the samples with the activated yeast cells was reduced by more than 74.3%.

In another specific embodiment of the invention, a method for producing yeast cells that decompose dimethylbenzene compounds, e.g., p-xylene, is provided. The
15 frequencies of the EM field(s) used to activate the yeast cells are in the range of 30.000 to 50.000 MHz, or 70.000 to 98.000 MHz including but not limited to 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.420 are cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM
20 fields in the order stated: 72 MHz at 93 mV/cm for 20 h; 80 MHz at 93 mV/cm for 20 h; 88 MHz at 93 mV/cm for 20 h; and 98 MHz at 93 mV/cm for 20 h.

The activity of the activated yeast cells towards dimethylbenzene compounds was determined by measuring the amounts of dimethylbenzene compounds the activated yeast cells can degrade. Two 100 liter samples each containing a dimethylbenzene
25 compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of p-xylene remaining in the samples were determined and compared by performing HPLC on the
30 samples. Comparing to samples with unactivated yeast cells, the amount of p-xylene in the samples with the activated yeast cells was reduced by more than 66.6%.

Yeast cell component that decomposes aldehyde compounds

In another specific embodiment of the invention, a method for producing yeast cells that decompose benzaldehyde and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 133-151 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, or 151 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.374 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 130 mV/cm for 30 h; 86 MHz at 130 mV/cm for 30 h; 94 MHz at 130 mV/cm for 30 h; 96 MHz at 130 mV/cm for 30 h.

The activity of the activated yeast cells towards benzaldehyde was determined by measuring the amounts of benzaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing benzaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of benzaldehyde remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of benzaldehyde in the samples with the activated yeast cells was reduced by more than 63.6%.

In yet another specific embodiment, a method for producing yeast cells that decompose propylaldehyde and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 145-162 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, and 162 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.414 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 103 mV/cm for 20 h; 88 MHz at 103 mV/cm for 20 h; 96 MHz at 103 mV/cm for 20 h; 98 MHz at 103 mV/cm for 30 h.

The activity of the activated yeast cells towards propylaldehyde was determined by measuring the amounts of propylaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing propylaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast

cells. After 24 hours, the amounts of propylaldehyde remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of propylaldehyde in the samples with the activated yeast cells was reduced by more than 73.8%.

5 In yet another specific embodiment, a method for producing yeast cells that decompose nenanthaldehyde compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 MHz or 100.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.503 were cultured at about 25-
10 30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 81 MHz at 90 mV/cm for 12 h, 85 MHz at 90 mV/cm for 12 h, 89 MHz at 90 mV/cm for 12 h, 94 MHz at 90 mV/cm for 12 h, 81 MHz at 157 mV/cm for 24 h, 85 MHz at 157 mV/cm for 24 h, 89 MHz at 157 mV/cm for 24 h, 94 MHz at 157 mV/cm for 24 h.

15 The activity of the activated yeast cells towards nenanthaldehyde was determined by measuring the amounts of nenanthaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing nenanthaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and
20 incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of nenanthaldehyde remaining in the samples were determined and compared by performing HPLC on the two samples. Comparing to samples with unactivated yeast cells, the amount of nenanthaldehyde in the samples with the activated yeast cells was reduced by more than 81.3% in 24 hours.

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Yeast cell component that decomposes halogen-substituted benzene compounds

In a specific embodiment, method for producing yeast cells that decompose halogen-substituted benzene compounds, e.g., m-dichlorobenzene, is provided. The
30 frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 163.000 to 183.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, and 183 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.483 were cultured at about 25-30°C in a culture
35 medium as described in Table 2 in the presence of a series of 4 EM fields in the order

stated: 72 MHz at 107 mV/cm for 20 h; 80 MHz at 107 mV/cm for 10 h; 90 MHz at 107 mV/cm for 30 h; 94 MHz at 107 mV/cm for 40 h.

The activity of the activated yeast cells towards dichlorobenzene was determined by measuring the amounts of dichlorobenzene the activated yeast cells can
5 degrade. Two 100 liter samples each containing dichlorobenzene concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24
10 hours, the amounts of dichlorobenzene remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dichlorobenzene in the samples with the activated yeast cells was reduced by more than 64.6%.

Yeast cell component that decomposes acetophenone and related compounds.

15 In yet another embodiment, a method for producing yeast cells that decompose acetophenone and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 175.000 to 191.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92,
20 94, 96, 97, 98, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, and 191 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.265 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 124 mV/cm for 20 h; 82 MHz at 124 mV/cm for 30 h; 90 MHz at 124 mV/cm for 40 h; 98 MHz at 124 mV/cm for 20 h.

25 The activity of the activated yeast cells towards acetophenone was determined by measuring the amounts of acetophenone the activated yeast cells can degrade. Two 100 liter samples each containing acetophenone at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and
30 incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of acetophenone remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of acetophenone compounds in the samples with the activated yeast cells was reduced by more than 75.5%.

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Yeast cell component that decomposes arsanilic acid and related compounds

In yet another embodiment, a method for producing yeast cells that decompose arsanilic acid and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 183.000 to 205.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, and 205 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.745 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 133 mV/cm for 30 h; 88 MHz at 133 mV/cm for 40 h; 92 MHz at 133 mV/cm for 30 h; 96MHz at 133 mV/cm for 30 h.

The activity of the activated yeast cells towards arsanilic acid was determined by measuring the amounts of arsanilic acid compounds the activated yeast cells can degrade. Two 100 liter samples each containing an arsanilic acid at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of arsanilic acid antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of arsanilic acid in the samples with the activated yeast cells was reduced by more than 75.5%.

Yeast cell component that decomposes roxarsone and related compounds

In another specific embodiment, a method for producing yeast cells that decompose roxarsone and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 114.000 to 128.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, and 128 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.173 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 110 mV/cm for 10 h; 92 MHz at 110 mV/cm for 10h; 78 MHz at 213 mV/cm for 30 h; 92 MHz at 213 mV/cm for 30 h.

The activity of the activated yeast cells towards roxarsone was determined by measuring the amounts of roxarsone the activated yeast cells can degrade. Two 100 liter samples each containing roxarsone concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of roxarsone remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of roxarsone in the samples with the activated yeast cells was reduced by more than 67.9%.

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Yeast cell component that decomposes furazolidonum compounds

In yet another specific embodiment, a method for producing yeast cells that decompose furazolidonum and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 200.000 to 220.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, and 220 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.397 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 74 MHz at 98 mV/cm for 30 h; 76 MHz at 98 mV/cm for 20 h; 86 MHz at 98 mV/cm for 30 h; 94 MHz at 98 mV/cm for 30 h.

The activity of the activated yeast cells towards furazolidonum was determined by measuring the amounts of furazolidonum the activated yeast cells can degrade. Two 100 liter samples each containing furazolidonum concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of furazolidonum remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of furazolidonum in the samples with the activated yeast cells was reduced by more than 81.4%.

Yeast cell component that decomposes Decoquinat

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In yet another specific embodiment, a method for producing yeast cells that decompose decoquinatone and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 213.000 to 229.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, and 229 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.452 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 112 mV/cm for 30 h; 82 MHz at 112 mV/cm for 30 h; 86 MHz at 112 mV/cm for 30 h; 94 MHz at 112 mV/cm for 20 h.

The activity of the activated yeast cells towards decoquinatone was determined by measuring the amounts of decoquinatone the activated yeast cells can degrade. Two 100 liter samples each containing decoquinatone concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of decoquinatone remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of decoquinatone in the samples with the activated yeast cells was reduced by more than 67.9%.

Yeast cell component that decomposes Trichlorophenol compounds

In yet another specific embodiment, a method for producing yeast cells that decompose trichlorophenol and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 220.000 to 250.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, and 250 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.100 were cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 74 MHz at 219 mV/cm for 30 h; 86 MHz at 219 mV/cm for 20 h; 96 MHz at 219 mV/cm for 30 h; 98 MHz at 219 mV/cm for 20 h.

The activity of the activated yeast cells towards trichlorophenol was determined by measuring the amounts of trichlorophenol the activated yeast cells can degrade. Two 100 liter samples each containing trichlorophenol concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated

yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of trichlorophonum remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of trichlorophonum in the samples with the activated yeast cells was reduced by more than 72.4%.

Yeast cell component that decomposes Dinitolmide

In a specific embodiment, method for producing yeast cells that decompose dinitolmide and related compounds is provided. Dinitolmide is 2-methyl-3,5-dinitrobenzamide and is also known as zoalene. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 220.000 to 250.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, and 250 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.189 are cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 202 mV/cm for 30 h; 82 MHz at 202 mV/cm for 30 h; 90 MHz at 202 mV/cm for 20 h; 96 MHz at 202 mV/cm for 20 h.

The activity of the activated yeast cells towards dinitolmide was determined by measuring the amounts of zoalene compounds the activated yeast cells can degrade. Two 100 liter samples each containing a dinitolmide concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of dinitolmide remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dinitolmide in the samples with the activated yeast cells was reduced by more than 72.4%.

Yeast cell component that removes ammonium compound (NH₄)

In a specific embodiment, a method for producing yeast cells that remove or reduce the level of ammonium compounds in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 660 to 680 MHz or 2160

to 2190 MHz, and preferably 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.614 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 662 MHz at 152 mv/cm for 18 h; 666 MHz at 152 mv/cm for 18 h; 672 MHz at 152 mv/cm for 18 h; 678 MHz at 152 mv/cm for 18 h; 662 MHz at 310 mv/cm for 25 h; 666 MHz at 310 mv/cm for 25 h; 672 MHz at 310 mv/cm for 35 h; 678 MHz at 310 mv/cm for 35 h.

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The activity of the activated yeast cells was determined by measuring the amounts of ammonium compounds removed by the activated yeast cells. The amount of ammonium compounds in the samples with the activated yeast cells was reduced significantly (>93.6%) compared to the sample containing unactivated yeast cells.

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Yeast cell component that removes nitrates and nitrites

In a specific embodiment, a method for producing yeast cells that remove or reduce the level of nitrates and nitrites in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 661.000 to 680.000 MHz including but not limited to 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, and 680 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.14 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 661 MHz at 126 mV/cm for 25 h; 665 MHz at 126 mV/cm for 25 h; 672 MHz at 126 mV/cm for 25 h; 676 MHz at 126 mV/cm for 25 h; 661 MHz at 196 mV/cm for 25 h; 665 MHz at 196 mV/cm for 25 h; 672 MHz at 196 mV/cm for 38 h; 676 MHz at 196 mV/cm for 38 h.

The activity of the activated yeast cells towards nitrates was determined by measuring the amounts of nitrates removed by the activated yeast cells. Two 100 liter samples each containing nitrates at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of nitrates remaining in the samples were determined and compared by performing HPLC

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on the samples. Comparing to samples with unactivated yeast cells, the amount of nitrates in the samples with the activated yeast cells was reduced by more than 69.7%

5 Yeast cell component that remove biologically available phosphorus

In a specific embodiment, a method for producing yeast cells that remove biologically available phosphorus, e.g., HPO_4^{2-} , H_2PO_4^- , etc., is provided. In a specific embodiment, a method for producing yeast cells that remove or reduce the level of nitrates and nitrites in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 80.000 to 440.000 MHz, preferably 86.000 to 120.000 MHz or 410.000 to 440.000 MHz including but not limited to 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, and 430 MHz. For example, Yeast cells of *Saccharomyces cerevisiae* strain AS2.620 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 98 MHz at 68 mv/cm for 24 h; 112 MHz at 68 mv/cm for 24 h; 108 MHz at 68 mv/cm for 24 h; 118 MHz at 68 mv/cm for 24 h; 98 MHz at 240 mv/cm for 24 h; 112 MHz at 240 mv/cm for 24 h; 108 MHz at 240 mv/cm for 42 h; 118 MHz at 240 mv/cm for 42 h.

The activity of the activated yeast cells towards available phosphorus was determined by measuring the amounts of available phosphorus the activated yeast cells can remove. Two 100 liter samples each containing available phosphorus concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of phosphorous remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of available phosphorus in the samples with the activated yeast cells was reduced by more than 65.8%.

Yeast cell component that decomposes Trichlorphon

In a specific embodiment, a method for producing yeast cells that decompose trichlorphon and related organophosphate pesticide compounds is provided. The

- frequencies of the EM field(s) used to activate the yeast cells are in the range of 1980.000 to 2020.000, and preferably 2000.000 to 2020.000 including but not limited to 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, and 2020 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.440 are cultured at about 25-30°C in a culture medium as described in
- 5 Table 2 in the presence of a series of 8 EM fields in the order stated: 2000 MHz at 125mv/cm for 10 h; 2004 MHz at 125 mv/cm for 10 h; 2009 MHz at 125 mv/cm for 24 h; 2018 MHz at 125 mv/cm for 24 h; 2000 MHz at 168 mv/cm for 10 h; 2004 MHz at 168mv/cm for 10 h; 2009 MHz at 168 mv/cm for 56 h; 2018 MHz at 168 mv/cm for 56 h.
- 10 The activity of the activated yeast cells towards trichlorphon was determined by measuring the amounts of trichlorphon the activated yeast cells can degrade. Two 100 liter samples each containing trichlorphon concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10⁷ cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C.
- 15 A control was included which did not contain any yeast cells. After 24 hours, the amount of trichlorphon remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of trichlorphon in the samples with the activated yeast cells was reduced by more than 10% in 48 hours.

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Yeast cell component that decomposes Dichlorvos

- In a specific embodiment, a method for producing yeast cells that decompose dichlorvos (DDVP) and related organophosphate pesticide compounds is provided. The
- 25 frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043, 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.443 are cultured at about 25-30°C in a culture medium as described in
- 30 Table 2 in the presence of a series of 8 EM fields in the order stated: 1993 MHz at 140 mV/cm for 24 h; 2023 MHz at 140 mV/cm for 24 h; 2083 MHz at 140 mV/cm for 24 h; 2103 MHz at 140 mV/cm for 24 h; 1993 MHz at 190 mV/cm for 24 h; 2023 MHz at 190 mV/cm for 24 h; 2083 MHz at 190 mV/cm for 56 h; 2103 MHz at 190 mV/cm for 56 h.

- The activity of the activated yeast cells towards dichlorvos was determined
- 35 by measuring the amounts of dichlorvos the activated yeast cells can degrade. Two 100 liter

samples each containing dichlorvos concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of

5 dichlorvos remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dichlorvos in the samples with the activated yeast cells was reduced by more than 67.5%.

Yeast cell component that decomposes Momocrotophos

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In a specific embodiment, a method for producing yeast cells that decompose momocrotophos and related insecticides is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043,

15 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.93 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 2998 MHz at 165 mV/cm for 24 h; 2033 MHz at 165 mV/cm for 24 h; 2058 MHz at 165 mV/cm for 24 h; 2113 MHz at 165 mV/cm for 24 h;

20 2998 MHz at 202 mV/cm for 56 h; 2033 MHz at 202 mV/cm for 56 h; 2058 MHz at 202 mV/cm for 24 h; 2113 MHz at 202 mV/cm for 24 h.

The activity of the activated yeast cells towards momocrotophos was determined by measuring the amount of momocrotophos the activated yeast cells can degrade. Two 100 liter samples each containing momocrotophos concentration of 100mg/L

25 were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of monocrotophos remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated

30 yeast cells, the amount of momocrotophos in the samples with the activated yeast cells was reduced by more than 73.4%.

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Yeast cell component that decomposes Dimethoate

In a specific embodiment, a method for producing yeast cells that decompose dimethoate and related insecticidal compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043, 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.379 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 1988 MHz at 195 mV/cm for 24 h; 2023 MHz at 195 mV/cm for 24 h; 2088 MHz at 195 mV/cm for 24 h; 2108 MHz at 195 mV/cm for 24 h; 1988 MHz at 277 mV/cm for 56 h; 2023 MHz at 277 mV/cm for 56 h; 2088 MHz at 277 mV/cm for 24 h; 2108 MHz at 277 mV/cm for 24 h.

The activity of the activated yeast cells towards dimethoate was determined by measuring the amount of dimethoate the activated yeast cells can degrade. Two 100 liter samples each containing dimethoate concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of dimethoate remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dimethoate in the samples with the activated yeast cells was reduced by more than 69.6%.

Yeast cell component that decomposes DDT

In a specific embodiment, a method for producing yeast cells that decompose DDT and related dilorinated organic insecticidal compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1420.000 to 1435.000 including but not limited to 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.415 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 1423 MHz at 75 mV/cm for 24 h; 1426 MHz at 75 mV/cm for 24 h; 1433 MHz at 75 mV/cm for 24 h; 1435 MHz at 75 mV/cm for 24 h; 1423 MHz at 146 mV/cm for 56 h; 1426 MHz at 146 mV/cm for 56 h; 1433 MHz at 146 mV/cm for 24 h; 1435 MHz at 146 mV/cm for 24 h.

The activity of the activated yeast cells towards DDT was determined by measuring the amount of DDT the activated yeast cells can degrade. Two 100 liter samples each containing DDT concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of DDT remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of DDT in the samples with the activated yeast cells was reduced by more than 78.5%.

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Yeast cell component that decomposes Toxaphene

In a specific embodiment, a method for producing yeast cells that decompose toxaphene and related chlorinated organic insecticidal compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1420.000 to 1435.000 including but not limited to 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.504 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 1420 MHz at 120 mV/cm for 24 h; 1426 MHz at 120 mV/cm for 24 h; 1431 MHz at 120 mV/cm for 24 h; 1434 MHz at 120 mV/cm for 24 h.

The activity of the activated yeast cells towards toxaphene was determined by measuring the amount of toxaphene the activated yeast cells can degrade. Two 100 liter samples each containing toxaphene concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of toxaphene remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of toxaphene in the samples with the activated yeast cells was reduced by more than 70.8%.

5.3. ODOR-REDUCING YEAST CELL COMPONENTS

The present invention also provides yeast cells that are capable of reducing the odor of solid waste, e.g., manures, sludge, and/or garbage. Without being bound by any theory, the inventor believes that the yeast cells of the invention are capable of reducing the

odor of solid waste by modifying, assimilating, or decomposing known and unknown compounds in the solid waste that are malodorous. However, it is not necessary to demonstrate that such compounds have been decomposed. It is sufficient so long as the odor is reduced as determined subjectively by a panel of subjects, after the yeast cells of the invention have been used.

According to the present invention, yeast cells that are capable of reducing the odor of solid waste are prepared by culturing the cells in the presence of an electromagnetic field in an appropriate culture medium. The frequency of the electromagnetic field for activating or enhancing this ability in yeasts can generally be found in the range of 2160 to 2380 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their ability to reduce the odor of solid waste by methods well known in the art.

The method of the invention for making the odor-reducing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(\text{NH}_4)_2\text{HPO}_4$, CaCO_3 , MgSO_4 , NaCl , and CaSO_4 .

It should be noted that the composition of the media provided in Table 38 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2 - 10^5 cell/ml, preferably 3×10^2 - 10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the

same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 2160 to 2380 MHz, and preferably in the ranges of 5 2160.000 to 2250.000 MHz or 2280.000 to 2380.000 MHz. The field strength of the EM field(s) is in the range of 25 to 300 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower 10 EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of 15 culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 80-320 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an 20 output amplitude of the EM wave in the range of 25-200 mV/cm. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the range of 250-300mV/cm. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing 25 process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m³, preferably 0.4 mol/m³. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the 30 culture by various methods known in the art, and stored at a temperature below about 0-4°C. The recovered yeast cells may also be dried and stored in powder form.

Any methods known in the art can be used to test the cultured yeast cells for their ability to reduce the odor of organic materials. The amount of malodorous chemicals such as hydrogen sulfide, ammonia, indole, p-cresol, skatol, and organic acids present in a 35 test sample of organic material can be determined by any methods known in the art,

including but not limited to gas phase chromatography, olfactometry, mass spectrometry, or the use of an odor panel.

For example, to determine the activity of the activated yeast cells towards an malodorous compound, mass spectrometry (e.g., VG micromass) can be used to measure the amounts of the malodorous compound in a test sample at various time point and under different incubation conditions. For example, a known amount of a malodorus compound (up to 100 mg per liter) is added to 10 liter of an aqueous extract of manure. Then, 0.1 ml of activated and unactivated yeasts (at least 10^7 cells/ml) are added to the 10 liter samples containing the compound, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of the malodorous compounds remaining in the extracts are determined and compared.

Yeast cell component that reduce odor caused by sulfur containing compounds

In one embodiment of the invention, a method for producing yeast cells that remove hydrogen sulfide and other related sulfur-containing or sulfhydryl (SH-) containing molecules is provided. Yeast cells that remove hydrogen sulfide and other related sulfur-containing or sulfhydryl (SH-) containing molecules can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.559 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2165 MHz at 240 mV/cm for 20 hr; 2175 MHz at 240 mV/cm for 20-60 hr; 2200 MHz at 240 mV/cm for 20 hr; 2235 MHz at 240 mV/cm for 20 hr.

The activity of the activated yeast cells towards sulfur-containing or sulfhydryl (SH-) compounds was determined by measuring the change in amount of hydrogen sulfides in the presence of the activated yeast cells. Two 100 liter samples each containing hydrogen sulfide concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of hydrogen sulfide remaining in the samples were determined and compared. Comparing to

samples with unactivated yeast cells, the amount of hydrogen sulfide in the samples with the activated yeast cells was reduced by more than 59.8%.

Yeast cell component that reduce odor caused by NH-containing containing compounds

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In another embodiment of the invention, a method for producing yeast cells that remove ammonia and related NH-containing compounds is provided. Yeast cells that remove ammonia and related NH-containing compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.423 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2160 MHz at 250 mV/cm for 20 hr; 2175 MHz at 250 mV/cm for 20 hr; 2210 MHz at 250 mV/cm for 20 hr; 2245 MHz at 250 mV/cm for 10 hr.

The activity of the activated yeast cells towards ammonia acid NH-containing compounds was determined by measuring the change in the amount of ammonia in the presence of the activated yeast cells. Two 100 liter samples each containing NH-containing compounds at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of ammonia remaining in the samples were determined and compared. Comparing to samples with unactivated yeast cells, the amount of ammonia in the samples with the activated yeast cells was reduced by more than 69.6%.

Yeast cell component that reduce odor caused by indole and other related compounds

In the invention, a method for producing yeast cells that decompose indole and other related compounds, such as skatol is provided. Yeast cells that decompose indole and other related compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.612 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2165 MHz at 240 mV/cm for 40 hr; 2180 MHz at 240 mV/cm for 20 hr; 2200 MHz at 240 mV/cm for 40 hr; 2220 MHz at 240 mV/cm for 20
5 hr.

The activity of the activated yeast cells towards indole and other related compounds was determined by measuring the amount of indole removed by the activated yeast cells. Two 100 liter samples each containing indole related compounds at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml
10 of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of indole remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of indole in the samples with the activated yeast
15 cells was reduced by more than 71.3%.

Yeast cell component that reduce odor caused by organic acids

In yet another embodiment of the invention, a method for producing yeast
20 cells that remove odorous organic acids, e.g., formic acid, acetic acid, propanoic acid, butyric acid, and other volatile fatty acids, is provided. Yeast cells that can reduce the odor of organic acids can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240,
25 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.53 are cultured at about 25-30°C in a culture medium as described in Table 42 in the presence of a series of 4 EM fields in the order stated: 2315 MHz at 290 mV/cm for 30 hr; 2335 MHz at 290 mV/cm for 10 hr; 2355 MHz at 290 mV/cm for 20 hr; 2375 MHz at 290 mV/cm for 10
30 hr.

The activity of the activated yeast cells towards organic acids was determined by measuring the change in the amounts of acetic acid in the presence of the activated yeast cells. Two 100 liter samples each containing organic acids concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated
35 yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and

incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of acetic acid remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of acetic acid in the samples with the activated yeast
5 cells was reduced by more than 89.4%.

Yeast cell component that reduce odor caused by aliphatic substituted amine

In yet another embodiment of the invention, a method for producing yeast
10 cells that remove or degrade aliphatic substituted amine, such as methylamine, dimethylamine, or trimethylamine thereby reducing the odor caused by such compounds, is provided. Yeast cells that remove or degrade such amines can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205,
15 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.541 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2160 MHz at 250 mV/cm for 20 hr; 2190 MHz at 250 mV/cm for 10 hr; 2210 MHz at 250 mV/cm for 40 hr; 2250 MHz at 250 mV/cm for 40
20 hr.

The activity of the activated yeast cells towards methyl-substituted amine was determined by measuring the amount of such amine in the presence of the activated yeast cells. Two 100 liter samples each containing methyl-substituted amine at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml
25 of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of methyl-substituted amines remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of methyl-substituted amines in the
30 samples with the activated yeast cells was reduced by more than 82.2%.

Yeast cell component that reduce odor caused by p-cresol and related compounds

In yet another embodiment of the invention, a method for producing yeast
35 cells that remove or degrade p-cresol and related compounds is provided. Yeast cells that

remove or degrade p-cresol and other related compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

5 For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.163 were cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2300 MHz at 98 mV/cm for 20 hr; 2370 MHz at 98 mV/cm for 15 hr; 2300 MHz at 250 mV/cm for 20 hr; 2370 MHz at 250 mV/cm for 30 hr.

 The activity of the activated yeast cells towards p-cresol and related
10 compounds was determined by measuring the change in the amounts of p-cresol and related compounds in the presence of the activated yeast cells. Two 100 liter samples each containing p-cresol at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was
15 included which did not contain any yeast cells. After 24 hours, the amounts of p-cresol remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of p-cresol in the samples with the activated yeast cells was reduced by more than 92.5%.

20 **5.4. PATHOGEN-SUPPRESSING YEAST CELL COMPONENTS**

 The present invention also provides yeast cells that are capable of suppressing the proliferation of pathogenic microorganisms that are present in solid waste. Typically, due to an abundance of nutrients present in solid waste for such pathogenic microorganisms, the numbers of pathogens increase rapidly over a period of time.
25 However, in the presence of the pathogen-suppressing yeasts of the invention, the numbers of pathogens in the treated solid waste remains unchanged, or decreases over time. Without being bound by any theory or mechanism, the inventor believes that the presence of the pathogen-suppressing yeasts in the solid waste creates an environment that is unfavorable for the growth of pathogenic microorganisms.

30 According to the invention, the ability of yeasts to affect/control the numbers of pathogens is activated or enhanced by culturing the yeasts in the presence of an electromagnetic field. The resulting pathogen-suppressing yeast cells are used as a component in the solid waste treatment compositions of the invention.

 The frequency of the electromagnetic field for activating or enhancing the
35 ability of yeasts to control the numbers of pathogenic microorganisms can generally be

found in the range of 30 MHz to 50 MHz. After sufficient time is given for the yeast cells to grow, the cells can be tested for their ability to affect/control the number of pathogens by methods well known in the art.

The method of the invention for making pathogen-suppressing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(\text{NH}_4)_2\text{HPO}_4$, CaCO_3 , MgSO_4 , NaCl , and CaSO_4 .

Table 4: Composition for a culture medium for Pathogen-Suppressing yeasts

20	Medium Composition	Quantity
	Soluble Starch	8.0g
	Sucrose	5g
	NaCl	0.2g
25	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
	$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	0.5g
	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.2g
	Peptone	1.5g
30	K_2HPO_4	0.5g
	Autoclaved water	400ml
	Pathogen extract	600ml

The pathogen extract for the culture medium is prepared by incubating 500g of pathogen-containing waste in about 600ml of warm water (at 35°C to 40°C) for 24 hours

at 30-37°C, and filtering the fluid to remove particulate matters. It should be noted that the composition of the media provided in Table 4 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of
5 media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2 - 10^5 cell/ml, preferably 3×10^2 - 10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series
10 of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 30.000 to 50.000, 500.000 to 650.000, and 1000.000
15 to 1150.000 MHz. The field strength of the EM field(s) is in the range of 20 to 200 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM
20 field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of
25 culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 144 - 272 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an
30 output amplitude of the EM wave in the range of 20-180 mV/cm. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the range of 200-350 mV/cm.

At the end of the culturing process, the pathogen-suppressing yeast cells may be recovered from the culture by various methods known in the art, and stored at about 0°C to 4°C. The pathogen-suppressing yeast cells may also be dried and stored in powder form.

The ability of the pathogen-suppressing yeasts to control the numbers of pathogens can be determined by any methods known in the art for enumerating microorganisms, such as optical density, plating out dilutions on solid media for counting, or counting individual cells under a microscope. Stains may be applied to distinguish or identify different strains or species of microorganisms present in a sample, or to determine their viability. When a range of pathogenic microorganisms are expected to be affected by the pathogen-suppressing yeasts, the numbers of more than one representative species of pathogenic microorganisms can be monitored to assess the performance of the pathogen-suppressing yeasts.

For example, samples of solid waste containing a known concentration of pathogenic microorganisms are cultured under the same conditions for a same period of time in the presence of different concentrations of pathogen-suppressing yeasts, and as negative control, the same strain of yeasts that have not been treated according to the culturing methods of the invention. A sample without any added yeast may also be included to determine the growth of pathogens under normal circumstances. The numbers of pathogens before and after the culture period are determined and compared.

A one liter culture containing at least 10^{10} cells of a pathogenic microorganism per ml is prepared. One ml of activated yeast cells (containing 2 to 5×10^7 yeasts per ml) is added to the one liter culture of pathogenic microorganism and incubated at 30°C for 24 hours. A control is included which contained unactivated yeast cells. The numbers of microorganisms in the respective culture is then determined and compared. The following are several examples of which a particular species of pathogenic bacteria was studied.

Yeast cell component that suppresses *Staphylococcus aureus*

In a specific embodiment of the invention embodiment, a method for producing yeast cells that suppress the growth of *Staphylococcus aureus* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.595 were cultured at about 25-30°C in a culture medium as described

in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for 12h; 43 MHz at 26 mV/cm for 12 h; 47 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 43 MHz at 150 mV/cm for 24 h; 47 MHz at 150 mV/cm for 24 h.

5 The activity of the activated yeast cells towards *Staphylococcus aureus* was determined by measuring the growth of *Staphylococcus aureus* in the presence of the activated yeast cells. *Staphylococcus aureus* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added
10 separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Staphylococcus aureus* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 2.6%.

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Yeast cell component that suppresses *Diplococcus pneumoniae*

In a specific embodiment, a method for producing yeast cells that suppress the growth of *Diplococcus pneumoniae* is provided. The frequencies of the EM field(s) used
20 to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain IFFI1021 were cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for
25 12h; 42 MHz at 26 mV/cm for 12 h; 49 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 42 MHz at 150 mV/cm for 24 h; 49 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *Diplococcus pneumoniae* was determined by measuring the growth of *Diplococcus pneumoniae* in the presence of the
30 activated yeast cells. *Diplococcus pneumoniae* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the
35 cell count of *Diplococcus pneumoniae* in the samples were determined by conventional

bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 3 %.

Yeast cell component that suppresses *Bacillus anthracis*

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In a specific embodiment, a method for producing yeast cells that suppress the growth of *Bacillus anthracis* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 20.000 to 45.000 MHz, including but not limited to 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 10 42, 43, 44, and 45 MHz. For example, yeast cells of *Bacillus anthracis* strain AS2.390 are cultured at about 25-30 °C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 24 MHz at 100 mv/cm for 24 h, 37 MHz at 100 mv/cm for 24 h, 40 MHz at 100 mv/cm for 24 h, 45 MHz at 100 mv/cm for 24 h, 24 MHz at 190 mv/cm for 24 h, 37 MHz at 190 mv/cm for 24 h, 40 MHz at 190 mv/cm for 24 h, 45 15 MHz at 190 mv/cm for 24 h.

The activity of the activated yeast cells towards *Bacillus anthracis* was determined by measuring the growth of *Bacillus anthracis* in the presence of the activated yeast cells. *Bacillus anthracis* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of 20 unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Bacillus anthracis* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples 25 with the activated yeast cells was reduced by 2.6 %.

Yeast cell component that suppresses *Mycobacterium tuberculosis*

In a specific embodiment, a method for producing yeast cells that suppress 30 *Mycobacterium tuberculosis* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.431 are cultured at about 25-30 ° in a culture medium as described in Table 4 in the presence of a series of 8 EM fields 35 in the order stated: 33 MHz at 26 mv/cm for 12 h; 36 MHz at 26 mv/cm for 12 h; 45 MHz

at 26 mv/cm for 12 h; 47 MHz at 26 mv/cm for 12 h; 33 MHz at 150 mv/cm for 24 h; 36 MHz at 150 mv/cm for 24 h; 45 MHz at 150 mv/cm for 24 h; 47 MHz at 150 mv/cm for 24 h.

The activity of the activated yeast cells towards *Mycobacterium tuberculosis* was determined by measuring the growth of *Mycobacterium tuberculosis* in the presence of the activated yeast cells. *Mycobacterium tuberculosis* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Mycobacterium tuberculosis* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 2.9 %.

15 Yeast cell component that suppresses *E. coli*

In a specific embodiment, a method for producing yeast cells that suppress the growth of *E. coli* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.561 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 34 MHz at 26 mV/cm for 12 h; 38 MHz at 26 mV/cm for 12 h; 49 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 34 43 MHz at 150 mV/cm for 24 h; 38 MHz at 150 mV/cm for 24 h; 49 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *E. coli* was determined by measuring the growth of *E. coli* in the presence of the activated yeast cells. *E. coli* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *E. coli* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with

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unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 48%.

Yeast cell component that suppresses *Salmonella*

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In a specific embodiment, a method for producing yeast cells that suppress the growth of *Salmonella* species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50
10 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.178 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 33 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for 12 h; 38 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 33 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 38 MHz at 150
15 mV/cm for 24 h.

The activity of the activated yeast cells towards *Salmonella* species was determined by measuring the growth of *Salmonella* in the presence of the activated yeast cells. *Salmonella* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts
20 cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Salmonella* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was
25 reduced by 62 %.

Yeast cell component that suppresses *Vibrio* species

In a specific embodiment, a method for producing yeast cells that suppress
30 *Vibrio* species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 500.000 to 550.000 MHz, including but not limited to 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, and 540 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.377 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a
35 series of 8 EM fields in the order stated: 521 MHz at 150 mv/cm for 24 h, 527 MHz at 150

mv/cm for 24 h, 531 MHz at 150 mv/cm for 24 h, 538 MHz at 150 mv/cm for 24 h, 521 MHz at 276 mv/cm for 24 h, 527 MHz at 276 mv/cm for 24 h, 531 MHz at 276 mv/cm for 24 h, 538 MHz at 276 mv/cm for 24 h.

The activity of the activated yeast cells towards *Vibrio* species was determined by measuring the growth of *Vibrio* species in the presence of the activated yeast cells. *Vibrio* species contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Vibrio* species in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 5.6 %.

15 Yeast cell component that suppresses *Shigella* species

In a specific embodiment, a method for producing yeast cells that suppress *Shigella* species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 600.000 to 650.000 MHz, including but not limited to 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, and 650 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.395 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 630 MHz at 180 mv/cm for 24 h, 636 MHz at 180 mv/cm for 24 h, 641 MHz at 180 mv/cm for 24 h, 649 MHz at 180 mv/cm for 24 h, 630 MHz at 314 mv/cm for 24 h, 636 MHz at 314 mv/cm for 24 h, 641 MHz at 314 mv/cm for 24 h, 649 MHz at 314 mv/cm for 24 h.

The activity of the activated yeast cells towards *Shigella* species was determined by measuring the growth of *Shigella* species in the presence of the activated yeast cells. *Shigella* species contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Shigella* species in the samples were determined by conventional bacteria cell counting

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method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 4.6 %.

Yeast cell component that suppresses *Clostridium botulinum*

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In a specific embodiment, a method for producing yeast cells that suppress *Clostridium botulinum* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1000.000 to 1050.000 MHz, including but not limited to 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023,
10 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1034, and 1035 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.432 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 1012 MHz at 180 mv/cm for 24 h, 1018 MHz at 180 mv/cm for 24 h, 1024 MHz at 180 mv/cm for 24 h, 1033 MHz at 180 mv/cm for 24 h, 1012 MHz at 323
15 mv/cm for 24 h, 1018 MHz at 323 mv/cm for 24 h, 1024 MHz at 323 mv/cm for 24 h, 1033 MHz at 323 mv/cm for 24 h.

The activity of the activated yeast cells towards *Clostridium botulinum* was determined by measuring the growth of *Clostridium botulinum* in the presence of the activated yeast cells. *Clostridium botulinum* contained in an extract from solid waste was
20 grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Clostridium botulinum* in the samples were determined by conventional
25 bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 5.1 %.

Yeast cell component that suppresses *Bacillus aerogenes capsulatus*

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In a specific embodiment, a method for producing yeast cells that suppress *Bacillus aerogenes capsulatus*. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1100.000 to 1150.000 MHz, including but not limited to 1100, 1101, 1102, 1103, 1104, 1105, 1106, 1107, 1108, 1109, 1110, 1111, 1112, 1113, 1114, 1115, 1116, 1117, 1118, 1119, and 1120 MHz. For example, yeast cells of *Saccharomyces*
35 *cerevisiae* strain AS2.432 are cultured at about 25-30°C in a culture medium as described in

Table 4 in the presence of a series of 8 EM fields in the order stated: 1102 MHz at 180 mv/cm for 24 h, 1106 MHz at 180 mv/cm for 24 h, 1113 MHz at 180 mv/cm for 24 h, 1117 MHz at 180 mv/cm for 24 h, 1102 MHz at 301 mv/cm for 24 h, 1106 MHz at 301 mv/cm for 24 h, 1113 MHz at 301 mv/cm for 24 h, 1117 MHz at 301 mv/cm for 24 h.

- 5 The activity of the activated yeast cells towards *Bacillus aerogenes capsulatus* was determined by measuring the growth of *Bacillus aerogenes capsulatus* in the presence of the activated yeast cells. *Bacillus aerogenes capsulatus* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Bacillus aerogenes capsulatus* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was
15 reduced by 6.2 %.

5.5 ADAPTATION

- In another embodiment of the invention, activated yeast cells prepared according to any one of Sections 5.1-5.10 can be further cultured as a mixture in the
20 presence of a sample of the solid waste which is to be treated. This optional process which improves the performance of the solid waste treatment compositions is described by way of an example as follows.

- An extract of the solid waste to be treated, such as manure or sludge, is prepared by mixing and soaking about 1000 g of poultry manure in 1000 to 3000 ml of
25 water for about 48 hours. The extract is then mixed with about 1000 g of dried manure (dry weight, i.e., less than 10% moisture) to form a suspension to which the yeast cells are added. At least 1 ml of yeasts which contains more than 5×10^7 cell/ml is added to the suspension. Depending on the number of strains of activated yeast cells used, up to about 50 ml of yeast cells can be added. If only a few strains are used, 5 to 10ml of yeast cells per
30 strain can be added. The process can be scaled up or down according to needs. The mixture of yeast and solid waste is cultured for about 120-280 hours in the presence of a series of electromagnetic fields. Each electromagnetic field has a frequency that, depending on the strains of yeast included, corresponds to one of the frequencies described in Sections 5.1-5.4. If many different strains of yeasts are used, a combination of the following five
35 frequency bands can be used : 20-50 MHz, 60-150 MHz, 400-700 MHz, 1400-1600 MHz,

2000-2500 MHz; each for about 24 to 56 hours. Generally, the yeast cells are subjected to an EM field strength in the range from 20mV/cm to 350mV/cm in this process.

The culture is incubated at temperatures that cycle between about 5°C to about 37°C. For example, in a typical cycle, the temperature of the culture may start at about 37°C and be kept at this temperature for about 1-2 hours, then adjusted to 26-30°C and kept at this temperature for about 2-4 hours, and then brought down to 5-10°C and kept at this temperature for about 1-2 hours, and then the temperature may be raised again to about 37°C for another cycle. The cycles are repeated until the process is completed. After the last temperature cycle is completed, the temperature of the culture is lowered to 3-4°C and kept at this temperature for about 5-6 hours. After the process, the yeast cells may be isolated and recovered from the medium by conventional methods, such as filtration. The adapted yeast cells can be stored under 4°C. An exemplary set-up of the culture process is depicted in Figure 2.

5.6 MANUFACTURE OF THE BIOLOGICAL COMPOSITIONS

The biological composition of the present invention can be produced by culturing yeast cells under appropriate conditions according to Section 5.1 to 5.4, and mixing the desired amounts of cultures of yeast cells. Since the biological composition is not immediately used to treat solid waste, the yeasts of the biological composition can be dried in a two-stage drying process. During the first drying stage, the yeast cells are dried in a first dryer at a temperature not exceeding 65°C for a period of time not exceeding 10 minutes so that yeast cells quickly become dormant. The yeast cells are then sent to a second dryer and dried at a temperature not exceeding 70°C for a period of time not exceeding 30 minutes to further remove water. After the two stages, the water content should be lower than 5%. It is preferred that the temperatures and drying times be adhered to in both drying stages so that yeast cells do not lose their vitality and functions. The dried yeast cells are then cooled to room temperature. The dried yeast cells may also be screened in a separator so that particles of a preferred size are selected. The dried cells can then be sent to a bulk bag filler for packing.

The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of

the invention. Indeed various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

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WHAT IS CLAIMED IS:

1. A method for treatment of solid waste comprising antibiotics, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the
5 yeast cells to degrade the antibiotics, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of
10 70 to 100 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of penicillin, chlortetracycline, oxytetracycline, doxycycline, tetracycline, streptomycin, kanamycin, erythromycin, spiramycin and bacitracin;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of
15 410 to 470 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of colistin, chloramphenicol, and cephalothin; or

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of
20 550 to 620 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of neomycin and novobiocin.

2. A method for treatment of solid waste comprising undesirable
25 chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following::

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of
30 52 to 98 MHz and a field strength of 8 to 300 mV/cm, which can degrade toluene, ethylbenzene, or trichlorophenol;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of
35 30 to 50 MHz or 70 to 98 MHz and a field strength of 8 to 250 mV/cm; and which can degrade dimethylbenzene compounds;

- (c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 133 to 151 MHz and a field strength of 8 to 250 mV/cm, and which can degrade benzaldehyde;
- 5 (d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 145 to 162 MHz and a field strength of 8 to 250 mV/cm, and which can degrade propylaldehyde;
- 10 (e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 100 MHz and a field strength of 8 to 250 mV/cm, and which can degrade nenanthaldehyde; and
- 15 (f) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 163 to 183 MHz and a field strength of 8 to 250 mV/cm, and which can degrade dichlorobenzene.

3. A method for treatment of solid waste comprising undesirable chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

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- (a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 175 to 191 MHz and a field strength of 8 to 250 mV/cm, and which can degrade acetophenone;
- 25 (b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 183 to 205 MHz and a field strength of 8 to 250 mV/cm, and which can degrade arsanilic acid;
- 30 (c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 114 to 128 MHz and a field strength of 8 to 250 mV/cm, and which can degrade is roxarsone;
- 35 (d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of

70 to 98 MHz or 200 to 220 MHz and a field strength of 8 to 250 mV/cm and which can degrade furazolidonum;

(e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 213 to 229 MHz and a field strength of 8 to 250 mV/cm and which can degrade decoquinat; and

(f) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 220 to 250 MHz and a field strength of 8 to 250 mV/cm, and which can degrade trichlorophonum or dinitomide.

4. A method for treatment of solid waste comprising undesirable chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 660 to 680 MHz or 2160 to 2190 MHz and a field strength of 25 to 300 mV/cm, and which can reduce the amount of ammonium compounds;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 661 to 680 MHz and a field strength of 8 to 250 mV/cm, and which can reduce the amount of nitrites or nitrates; or

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 86 to 120 MHz or 410 to 430 MHz and a field strength of 8 to 250 mV/cm, and which can reduce the amount of phosphates.

5. A method for treatment of solid waste comprising undesirable chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1980 to 2118 MHz and a field strength of 25 to 300mV/cm, and which can

degrade undesirable chemical selected from the group consisting of trichlorphon, dichlorvos, momocrotophos and dimethoate;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1420 to 1435 MHz and a field strength of 25 to 300mV/cm, and which can degrade DDT or toxaphene.

6. A method for reducing the odor of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of odorous molecules in the solid waste, wherein said yeast are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 2160 to 2380 MHz and a field strength of 25 to 300 mV/cm, and said odorous molecules are selected from the group consisting of hydrogen sulfide, ammonia, indole, skatol, acetic acid, methylamine, and p-cresol.

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7. A method for treatment of solid waste comprising pathogenic bacteria, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to suppress the growth of pathogenic bacteria in the solid waste, wherein said plurality of yeast cells comprises at least one of the following:

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(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 20 to 50 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of *Staphylococcus aureus*, *Diplococcus pneumoniae*, *Bacillus anthracis*, *Mycobacterium tuberculosis*, *Salmonella* species, or *E. coli*;

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(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 500 to 550 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of *Vibrio* species;

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(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 600 to 650 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of *Shigella* species;

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(d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of

1000 to 1050 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of *Clostridium botulinum*; and

- 5 (e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1100 to 1150 MHz and a field strength of 20 to 50 mV/cm, and which can suppress the growth of *Bacillus aerogenes capsulatus*.

8. A method for treatment of solid waste comprising adding a biological composition to the solid waste, said biological composition comprising at least one of the following yeast cell components:

- 10 (a) a first yeast cell component comprising a plurality of yeast cells that degrade antibiotics in solid waste, said first yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range selected from the group consisting of 70 to 100 MHz, 410
15 to 470 MHz, and 550 to 620 MHz and a field strength of 8 to 250 mV/cm;

- (b) a second yeast cell component comprising a plurality of yeast cells that degrade undesirable chemicals in solid waste, said second yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range selected from the group consisting of
20 30 to 100 MHz, 70 to 280 MHz, 410 to 430 MHz, 660 to 680 MHz and 1980 to 2210 MHz and a field strength of 8 to 250 mV/cm;

- (c) a third yeast cell component comprising a plurality of yeast cells that reduce the amount of odorous molecules in solid waste, said third yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of
25 electromagnetic fields having a frequency in the range of 2160 to 2380 MHz and a field strength of 25 to 300 mV/cm;

- (d) a fourth yeast cell component comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in the solid waste, said fourth yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series
30 of electromagnetic fields having a frequency in the range selected from the group consisting of 30 to 50 MHz, 500 to 550 MHz, 600 to 650 MHz, 1000 to 1050 MHz, and 1100 to 1150 MHz and a field strength of 20 to 350 mV/cm; and

allowing the yeast cells in the yeast cell component(s) to reduce the amount of antibiotics, undesirable chemicals, odorous compounds and pathogenic bacteria in the solid
35 waste.

9. The method of claim 8, wherein the biological composition comprises the yeast cell components of (a), (b), (c) and (d).
10. The method of claim 8 wherein said yeast cells are cells of a species
5 of yeast selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Saccharomyces delbrueckii*, *Saccharomyces exiguus*, *Saccharomyces fermentati*, *Saccharomyces logos*, *Saccharomyces mellis*, *Saccharomyces microellipsoides*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Saccharomyces rouxii*, *Saccharomyces sake*, *Saccharomyces uvarum* Beijer, *Saccharomyces willianus*, *Saccharomyces ludwigii*,
10 *Saccharomyces sinenses*, and *Saccharomyces carlsbergensis*.
11. The method of claim 8 wherein said yeast cells are *Saccharomyces cerevisiae* cells.
12. The method of claim 11 wherein said biological composition
15 comprises dried yeast cells, and about 300 to 600g of the biological composition is added per cubic meter of solid waste.
13. The method of claim 11 wherein prior to adding said dried yeast cells
20 to said solid waste, said dried yeast cells are mixed with water in the ratio of about 1000 g yeast cells to about 30 liters, and incubated for about 12 to 24 hours.
14. A composition comprising a plurality of yeast cells that degrade antibiotics in solid waste, wherein said plurality of yeast cells is prepared by a method
25 comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range selected from the group consisting of 70 to 100 MHz, 410 to 470 MHz, and 550 to 620 MHz and (ii) a field strength of 8 to 250 mV/cm.
15. A composition comprising a plurality of yeast cells that degrade undesirable chemicals in solid waste, wherein said plurality of yeast cells is prepared by a method comprising culturing yeast cells in an electromagnetic field or a series of
30 electromagnetic fields having (i) one or more frequencies in the range selected from the group consisting of 30 to 100 MHz, 70 to 280 MHz, 410 to 430 MHz, 660 to 680 MHz and
35 1980 to 2210 MHz and (ii) a field strength of 8 to 250 mV/cm.

16. A composition comprising a plurality of yeast cells that reduce the odor of solid waste, wherein said plurality of yeast cells is prepared by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having
5 (i) one or more frequencies in the range of 2160 to 2380 MHz and (ii) a field strength of 25 to 300 mV/cm;

17. A composition comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in solid waste, wherein said plurality of yeast cells is prepared
10 by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range selected from the group consisting of 30 to 50 MHz, 500 to 550 MHz, 600 to 650 MHz, 1000 to 1050 MHz, and 1100 to 1150 MHz and (ii) a field strength of 20 to 350 mV/cm.

15 18. The composition of claim 14, 15, 16, or 17, wherein said yeast cells are cells of a species of yeast selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Saccharomyces delbrueckii*, *Saccharomyces exiguus*, *Saccharomyces fermentati*, *Saccharomyces logos*, *Saccharomyces mellis*, *Saccharomyces microellipsoides*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Saccharomyces rouxii*,
20 *Saccharomyces sake*, *Saccharomyces uvarum* Beijer, *Saccharomyces willianus*, *Saccharomyces ludwigii*, *Saccharomyces sinenses*, and *Saccharomyces carlsbergensis*.

19. The composition of claim 14, 15, 16, or 17, wherein said yeast cells are dried yeast cells.
25

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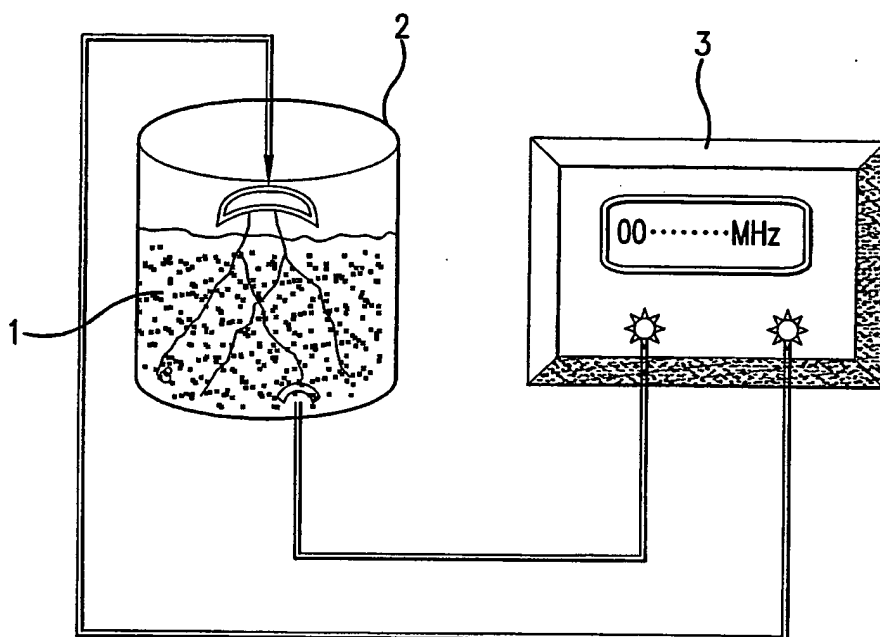


FIG.1

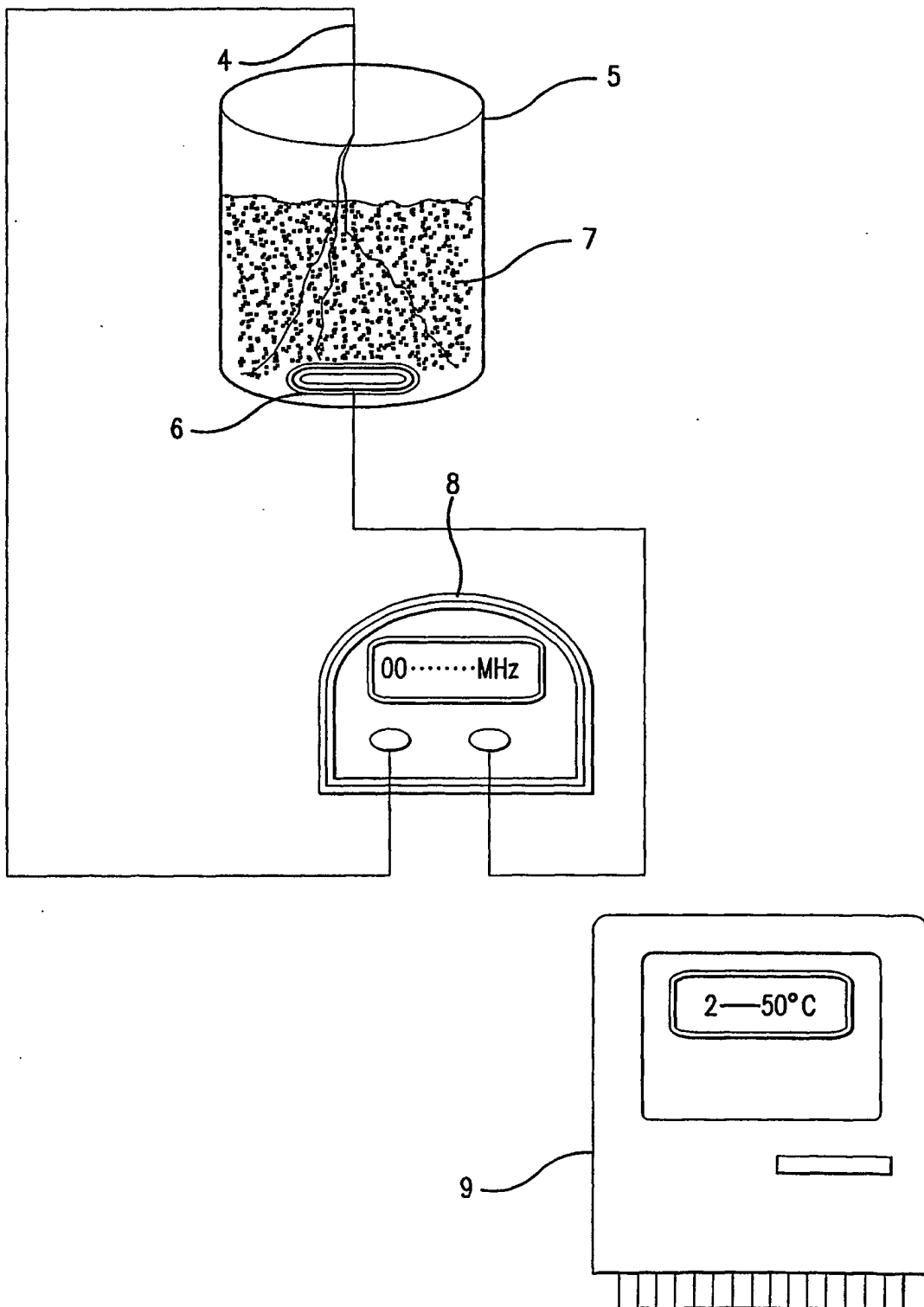


FIG.2

INTERNATIONAL SEARCH REPORT

Interr. Application No

PCT/GB 02/00915

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N13/00 C12N1/16 C12N1/04 C12P1/02 A62D3/00
B09B3/00 //(C12P1/02, C12R1:645)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12P C12R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"Saccharomyces cerevisiae Meyen ex Hansen" CHINA CATALOGUE OF CULTURES/CHINA COMMITTEE OF CULTURE COLLECTION FOR MICROORGANISMS (CCCCM), 'Online! 24 April 1996 (1996-04-24), XP002223046 Retrieved from the Internet: <URL:http://www.im.ac.cn/database/YEAST/y122.htm> 'retrieved on 2002-12-02! the whole document	14-19
X	WO 87 02705 A (SWEENEY GEORGE WILLIAM JR) 7 May 1987 (1987-05-07) the whole document	1-13
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

3 December 2002

Date of mailing of the international search report

20/12/2002

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Dumont, E

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/00915

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PICHKO V B ET AL: "Electromagnetic stimulation of productivity of microorganisms and its mechanisms." PRIKLADNAYA BIOKHIMIYA I MIKROBIOLOGIYA, vol. 32, no. 4, 1996, pages 468-472, XP009001867 ISSN: 0555-1099 abstract ---	1-13
X	BINNINGER DAVID M ET AL: "Effects of 60Hz AC magnetic fields on gene expression following exposure over multiple cell generations using Saccharomyces cerevisiae." BIOELECTROCHEMISTRY AND BIOENERGETICS, vol. 43, no. 1, 1997, pages 83-89, XP002223047 ISSN: 0302-4598 the whole document especially abstract; discussion ---	1-13
X	FR 2 222 433 A (GIANESSY MATILDE) 18 October 1974 (1974-10-18) the whole document ---	1-13
X	WO 95 04814 A (INT TLB RES INST INC) 16 February 1995 (1995-02-16) page 11, line 25 - line 38 page 16, line 1 - line 10 ---	1-13
X	US 3 870 599 A (AZAROWICZ EDWARD N) 11 March 1975 (1975-03-11) the whole document ---	15, 19
X	DATABASE WPI Week 198439 Derwent Publications Ltd., London, GB; AN 1984-242742 '39! XP002223049 "Yeast strain Exophiala Nigrum R-11 - removes phenol(s) and lignin from aq. effluent(s) " & SU 1 071 637 A (NII BIOLOG PRI IR G UNIV IM A), 7 February 1984 (1984-02-07) abstract ---	15
E	WO 02 20431 A (ULTRA BIOTECH LTD) 14 March 2002 (2002-03-14) page 8, line 1 -page 13, line 15; claims 1-51 ---	1-19

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/00915

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>PONNE C T ET AL: "Interaction of electromagnetic energy with biological material--relation to food processing" RADIATION PHYSICS AND CHEMISTRY, ELSEVIER SCIENCE PUBLISHERS BV., AMSTERDAM, NL, vol. 45, no. 4, 1 April 1995 (1995-04-01), pages 591-607, XP004051598 ISSN: 0969-806X page 591, column 2, paragraph 3 -page 601, column 2, paragraph 3 -----</p>	1-19

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1, 14 (complete) and 8-13, 18,19 (partial)

a method for treatment of solid waste comprising antibiotics, comprising adding to the solid waste yeast cells cultured in an electromagnetic field of specific frequency and field strength; a composition comprising such yeasts.

2. Claims: 2, 3, 4, 5, 15 (complete) and 8-13, 18, 19 (partial)

a method for treatment of solid waste comprising chemicals including herbicides, pesticides and fertilizer-related pollutants, comprising adding to the solid waste yeast cells cultured in an electromagnetic field of specific frequency and field strength; a composition comprising such yeasts.

3. Claims: 6, 16 (complete) and 8-13, 18, 19 (partial)

a method for reducing the odor of solid waste, comprising adding to the solid waste yeast cells cultured in an electromagnetic field of specific frequency and field strength; a composition comprising such yeasts.

4. Claims: 7,17 (complete) and 8-13, 18, 19 (partial)

a method of treatment of solid waste comprising pathogenic bacteria, comprising adding to the solid waste yeast cells cultured in an electromagnetic field of specific frequency and field strength; a composition comprising such yeasts.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/00915

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 02/00915

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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WO 0220431	A	14-03-2002	US 6416983 B1 WO 0220431 A1 AU 7020300 A WO 02070436 A2	09-07-2002 14-03-2002 22-03-2002 12-09-2002